



AGRICULTURAL RESEARCH INSTITUTE

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# HILGARDIA

*A Journal of Agricultural Science*

PUBLISHED BY THE

*California Agricultural Experiment Station*

VOLUME VII

JUNE, 1932, TO SEPTEMBER, 1933

With 12 Plates and 132 Text Figures

UNIVERSITY OF CALIFORNIA  
BERKELEY, CALIFORNIA

1933



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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

JUNE, 1932

No. 1

## VARIATIONS IN CITRUS SEEDLINGS AND THEIR RELATION TO ROOTSTOCK SELECTION<sup>1,2</sup>

H. J. WEBBER<sup>3</sup>

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<sup>1</sup> Received for publication May 3, 1932.

<sup>2</sup> Paper No. 263, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

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## INTRODUCTION

Although the question of securing the best rootstocks to use in citrus propagation has for many years attracted the attention of growers, experimentation on the subject has been very limited. The earliest general publication on citrus stocks in America, that of Van Deman (1891), is a summary of the observations and studies made on groves in Florida and is not based on comparative experiments. Mills (1902) has described the results of certain experiments conducted by the California Experiment Station at Pomona, California; and Bonns and Mertz (1916), the results of a series of comparative experiments made at the Citrus Experiment Station at Riverside.

A carefully planned and executed experiment was also carried out for a limited time by Taber (1904) at Glen St. Mary, Florida, with certain varieties propagated on Trifoliolate orange, sour orange, and sweet orange. The experiment was designed primarily to determine the comparative value of the cold-resistant Trifoliolate orange as a stock.

As a result of these studies and experiments and of the cumulative understanding of growers derived from long experience, certain stocks have come to be commonly used, and success in general has been achieved with them. It is well recognized, however, that the problems connected with rootstocks are poorly understood, and there is little evidence to justify a conclusion that the species and varieties now used as stocks are the best available. A fair appreciation of the value of sour orange, sweet orange, lemon, grapefruit, and certain other species as stocks has been acquired, but until recently no attention has been directed to variations within these species and the influence of such variations on the fruit or scion variety. Until recently this was also the case in the propagation of all other commercial fruits, such as the apple and pear.

The experiments herein reported, which were started in 1914 (Webber, 1919 and 1920), resulted in directing attention to the great variability among seedlings of the same species or stock type, and to the probable influence of such variation on the uniformity of the orchard trees produced. The present paper will outline the results obtained with these experiments up to the present time, and will discuss briefly the influence that the findings may have in improving nursery methods in the future.

It has been claimed for certain fruits, such as the apple (Swarbrick and Roberts, 1927; and Roberts, 1929), that the scion variety dominates the scion-stock combination, determining the character and form of the root produced. It is to be regretted that no thorough study of this point has as yet been made with citrus, owing largely to the almost universal practice of "balling" nursery trees in transplanting, a practice which does not permit an examination of the roots. In such trees as have been examined nothing has been observed to indicate that the scion has any material effect in changing the characteristic branching of the root, whether budded low or high. As an illustration, the examination of a considerable number of orchard trees of all ages from 6 to 75 years, that has been made from time to time, has shown conclusively that the tendency of the sour orange to form a distinct taproot is not visibly modified by the scion variety; and that the sweet orange, which normally shows a weak taproot development, exhibits this character when used as a stock, regardless of the scion variety. Of course it is true that some other scion variety than those observed might exert a profound influence on the stock, and careful studies may show influences that are not now suspected.

The writer's observations, though limited in extent, apparently confirm the results obtained by Amos, Hatton, Hoblyn, and Knight (1930), and Vyvyan (1930) in their studies of the effect of scion on root in apples, where the roots of the stock type were found to retain their characteristic branching regardless of the scion variety used or the height of the insertion of the scion. The size of the root, however, was distinctly influenced, and this is also the case with citrus.

The writer has observed numerous cases where the size of the root system was doubled or quadrupled by the reaction of different scions. Very remarkable influences of this sort have also been recorded in several stionic combinations in citrus by Brown (1920) in India.

Recently in experiments with the Trifoliate orange used as a stock with various orange, mandarin, lemon, and grapefruit varieties, Tanaka (1931) has described certain influences on the stock caused by the scion variety. He states "Generally speaking the subterranean part of sweet-orange top is deep rooted (branches narrow angled), while that of lemon top is shallow rooted (branches broad angled). The color of Trifoliate root used for the lemon stock shows lighter coloration than when other scions are employed for the top."

The evidence presented in this paper also points to the conclusion that the size and vigor of the orchard tree is considerably influenced



by the character of the seedling used as a stock, and apparently justifies the adoption of some method of stock selection.

That more complex influences also occur is shown by the reaction on the normal soluble magnesium content in the ash of the bark of the stock as effected by that of the scion. Citrus species differ in the normal magnesium content in the bark, and a normally high-magnesium-content scion has been shown to increase that of a low-content stock, while a normal low-magnesium content of the scion type tends to depress that of a high-content stock (Haas and Halma, 1929).

The writer's studies and experiments have revealed many cases where there is a mutual influence between the stock and scion. It is clear that these influences require study to determine the extent of the influence for each variety and stock combination, in order that orchards may be planned on a safe and sure foundation.

The influence of the stock on the scion, and vice versa, may in general be considered as similar to an environmental reaction. Each of the two distinct portions of the tree retains its individual or genetic characteristics, but these may be modified in expression by the changed stionic conditions, much as they might be modified by a change of environment. These changes are usually quantitative variations such as changes in size of plant or fruit, size of crop, longevity, and density of color. The characters that are changed by the reactions between stock and scion, for simplicity of expression are here designated as *stionic variations* or *stionic reactions*.<sup>4</sup>

The present paper deals mainly with the stionic reactions caused by the use of seedling stocks of the same species but of different sizes and types. An attempt is made to answer the following questions: (1) Do all seedlings of the same species or even of the same variety, when budded with the same fruit variety, produce orchard trees of standard size and character? (2) If not, is there any means of segregating the good seedlings from the bad?

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<sup>4</sup> For the sake of clarity and brevity in this discussion, several new words coined by the writer are introduced, which require explanation:

*Stion*—any plant or tree composed of a stock and scion growing in combination. This term is used regardless of the method employed in propagation, i.e., budding, grafting, inarching, etc. It is formed by combining the first two letters of the word *stock* with the last three letters of *scion*.

*Stionic*—pertaining to a stion.

*Stionic variation*—a variation caused by the reaction between stock and scion.

*Budling*—a young, budded nursery tree.

## EXPERIMENTAL METHODS AND MATERIALS

In the several experiments to be reported here, care was used to treat the plants in each experiment as uniformly as possible in order to reduce the variation to a minimum, except as caused by the different stocks used.

Considerable doubt existed in the beginning as to what records should be made that would best indicate tree size at various stages of growth. The size measurements of the seedlings used were diameter or circumference of trunk taken 3 to 4 inches above the ground, and the greatest height attained by the plant. No feasible method of measuring the top volume of a small seedling was found. It may be worthy of note here that it has since been regretted that the weight of the top of each seedling was not recorded when the top was cut off to force the bud, for such weights would probably have been very accurate indicators of the comparative size of the nursery trees.

The same measurements which were taken for the seedlings were also taken for the budlings, namely, diameter or circumference of trunk, and maximum height. The diameter or circumference of the stock was taken at the smallest point between the soil and the bud union, and that of the scion trunk at a point 2 inches above the bud, unless otherwise stated. These measurements of the budded nursery trees were made in the early spring immediately preceding the transplanting of the budlings to the orchard, and were thus the measurements for the 1-year-old nursery trees. The height measurements of citrus nursery trees, whether of the seedlings or of the budlings, have been found in most cases to be a very poor indicator of size and thus have not been used in this paper.

The size and vigor of the orchard trees were judged by trunk size as indicated by circumference measurements, volume of top, and total yields of fruit. The trunk size was determined by circumference measurements of the stock trunk at the smallest point between the soil and the bud union, and that of the scion trunk by circumference measurements at a point between 4 and 6 inches above the bud union. The top volume was determined by the use of a fumigation tent placed over the tree; the measurements were carefully recorded and the volume obtained by the Woglum formula (Woglum, 1909, p. 25). The yield of each tree under experiment is determined by weighing the fruit in the field and is recorded each year in pounds per tree.

In the statistical calculations, area of trunk cross section is used rather than diameter or circumference, and is of course obtained from the diameter or circumference records. Such measurements have been taken uniformly in centimeters and square centimeters, while volume measurements have been taken in cubic feet, and the yields in pounds. As all students are familiar with both systems of weights and measures, it has not seemed worth while to transpose the figures into a uniform system.

In the discussion presented here the nomenclature used is that prevalent in citrus sections of the United States and is based on that given by W. T. Swingle in Bailey's *Standard Cyclopaedia of Horticulture*. The common names of the species are used mostly and if a special variety is used its generally recognized name is given. The following are the principal common names used with their equivalent botanical names: sweet orange (*Citrus sinensis* Osbeck); sour orange (*C. aurantium* Linn.); grapefruit (*C. maxima* Merrill); Rough lemon (variety of *C. limonia* Osbeck); Trifoliate orange (*Poncirus trifoliata* Raf.).

## VARIATION, APOGAMY, AND POLYEMBRYONY IN CITRUS

Before proceeding to the discussion of the experiments it will probably clarify the problems involved to discuss the nature of the variation occurring among citrus seedlings and the influence that is introduced by the very general occurrence of the phenomena of apogamy and polyembryony.

### VARIATIONS AMONG NURSERY SEEDLINGS

During the last 40 years, the sour orange has been more extensively used as a rootstock in California, Florida, and Mediterranean countries than any other *Citrus* species, and therefore, the study of variations in the seedlings of this species is of particular interest from the stock standpoint. In 1915, at an early stage in the present investigations, the examination of a commercial nursery near Whittier, California, of sour-orange seedlings which were of the size and age for budding revealed the presence of what appeared to be a considerable number of different types. All apparently were sour-orange seedlings but certain individuals exhibited different characters of size, foliage, branching, and general habit from the prevailing and apparently normal type exhibited by the other seedlings in the same

nursery. About 25 different seedlings that seemed to vary in some morphological character from the general type were chosen for study, and buds were taken from each to propagate and test the type. At the same time one particularly large and vigorous seedling of an apparently normal type was also selected for comparison. Two trees of each of the types chosen were propagated on Rough-lemon stocks. The budlings of these were grown at the Citrus Experiment Station and in the spring of 1917 were planted in a row in the experimental orchard (field I, block A, row 16) adjacent to the experimental rows of large, medium, and small trees described in the following section.

The trees propagated from these variant<sup>5</sup> seedlings which in the nursery had not appeared to be very widely different from each other or from the normal type of the sour orange, as they grew older exhibited markedly different characters in size, branching, foliage, and fruits. The trees are now 15 years old (17 years from the bud) and they still retain in equal degree their characteristic differences as described in earlier publications (Webber, 1920 and 1920a). Several of the trees are veritable dwarfs, the tops at 15 years of age being only about 4 feet high, densely branched, and "scrubby" in appearance, while others are of various sizes up to nearly standard (fig. 1). Some were so weak and aberrant that they lived a few months only. The seedling that was chosen as representing what appeared to be a good standard of the prevailing or normal type in the nursery was propagated and grown along with the others. It proved to be a vigorous grower and is to be considered as representing an excellent strain of the sour orange.<sup>6</sup>

The range of differences in branching, foliage, and fruit characters exhibited by the trees propagated from these variant seedlings is in some cases as great as that found between diverse species of *Citrus*, and yet all were taken from one comparatively small sour-orange nursery and were apparently direct derivatives from the sour orange.

The examination of other orange nurseries in various parts of the state demonstrated the fact that similar variations among the seedlings occurred commonly. The variations were present in about equal numbers in every sour-orange nursery examined and what appeared to be

<sup>5</sup> The term "variant" is used here to designate any seedling that is different from the normal or ordinary type in a certain progeny, in any easily recognizable character.

<sup>6</sup> In later experiments, seedlings from one tree of this strain or clon have been used as representing a selected standard strain of the sour orange. "Standard" has become a varietal name for this clon of the sour orange, as a result of the continuous use of the term.

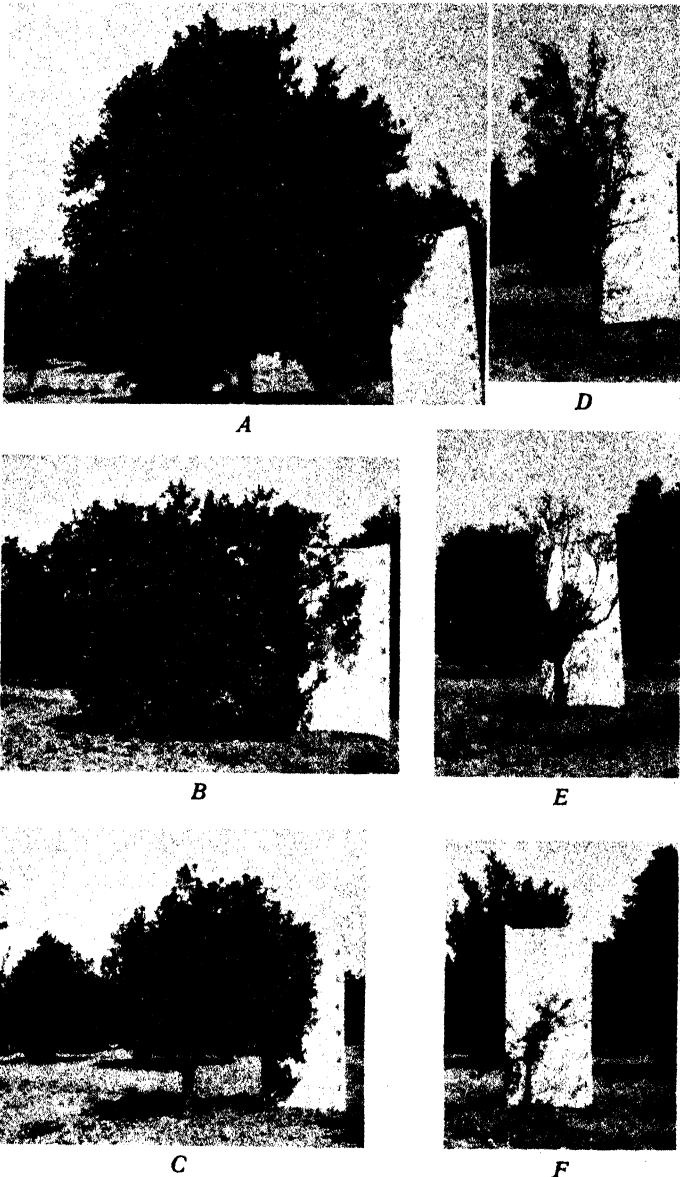


Fig. 1. Types of sour orange propagated from variant seedlings found in nursery at Whittier, California. *A*, selected normal type, the standard; *B*, medium large size, spreading top; *C*, medium size, no oil glands; *D*, erect columnar top, long narrow leaves; *E*, small weak type; *F*, extreme dwarf but still living. All are 15 years old from date of setting in orchard and are on Rough-lemon stocks.

similar variants were also found among nursery seedlings of the sweet orange, grapefruit, and Rough lemon.

In most nurseries, at the time these preliminary observations were being made, such seedlings as were deemed too small when the first budding was done were left and budded later when they had reached the proper size. Budlings that were too small when the main lot was dug and sold were held an extra year or more until they reached salable size. Could it be that these small seedlings, which apparently are mainly variants, would produce equally good orchard trees when budded with good standard fruit varieties, or might it be that the poorly growing trees that were to be found in many orchards were inadvertently propagated on such variant seedling stocks? No experiments had been made to determine what influence such variant types of seedlings had on the scion, and this was evidently an important point to determine. Experimental data obtained from tests of such seedlings will be given in a later section of this paper.

#### APOGAMY AND POLYEMBRYONY

Of even greater importance than the occurrence of these variants was the fact that a large proportion of the seedlings grown in any nursery from seed derived from the same source, presented a prevailing dominant type that was usually exhibited by from 60 to 90 per cent of the total population. The occurrence of this dominant type in such a large proportion of the seedlings is evidently due to the prevalence of apogamic reproduction in the various *Citrus* species and varieties.

In the great majority of plants, seed production is from necessity preceded by the pollination of the stigma followed by the fecundation of the single egg cell and the development of a single embryo from the egg cell. The various species and varieties of citrus, however, have evolved the ability, through means of the phenomenon termed "apogamy,"<sup>7</sup> to produce viable embryos in the seed which have no direct relation to the regular egg apparatus and fecundation.

According to the investigations of Strasburger (1878) and Osawa (1912), this is accomplished by the specialization of certain cells or groups of cells in the body of the ovary (nucellus) of the mother near the wall of the embryo sac. These cells become highly protoplasmic,

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<sup>7</sup> "Apogamy" is used here in its general sense as referring to the production of embryos in the seed without fecundation, from diploid cells of the mother plant. Reproduction through such embryos is equivalent to vegetative propagation and gives rise to clones, the individuals of which are genetically homogeneous.

grow and divide more rapidly than the neighboring cells, and finally form masses of tissue which push out into the embryo sac and form embryos so nearly like those which develop from the egg cells proper that the two types of embryos cannot be distinguished one from the other in the seed.

Since the apogamic embryos originate from the somatic tissue of the mother and are not preceded by a reducing division and fecundation, they naturally carry the full diploid chromosome complement direct from the mother and transmit the same heritage as the mother type. It would be expected, therefore, that they would reproduce seedlings of the same type as the mother unless some irregularity occurs in an occasional cell division, and such irregularities are not common.

While the ovaries of citrus contain (except very rarely) only one egg cell each, and thus one sexually developed embryo, this apogamic development commonly leads to the formation of several embryos (usually from 2 to 4 and occasionally as high as 10 or 12) in each seed (polyembryony). All of these are somatic in origin except that one which comes from the egg cell following fecundation. It is also an important phenomenon that apparently this one sexual embryo frequently fails to develop, owing to lack of fecundation, crowding, or some other cause, in which case all of the embryos of a seed are apogamic.

In 1900 Webber pointed out the difficulties that this phenomenon introduces into the study of citrus hybrids, where a large percentage of the seedlings that develop from carefully crossed and guarded flowers are of apogamic origin. The seedlings from apogamic embryos cannot be distinguished readily in early stages from the true hybrids unless the parents differed markedly in some character which, combined in the hybrid, results in some distinctive character of foliage that would enable the hybrids to be recognized. In hybrids of parents with similar foliage and plant-body characters the true hybrids cannot be distinguished with certainty until they bear fruit, thus necessitating the expense of growing large numbers to secure a few hybrids. Here apogamy is a distinct disadvantage.

In citrus culture the main influence of apogamy is likely to be found in its relation to the problem of securing rootstocks of uniform character. During the last 10 years attention has been focused on the very great importance of the character of the rootstocks used in horticultural propagations. It has become increasingly evident that the genetic variation in seedlings used as stocks is to be considered

responsible for much of the variation in tree size and production exhibited in orchards. Therefore, in the propagation of citrus, as is the case with other orchard fruits, the great desideratum is the availability of rootstocks that are known to possess the same heritage and to react similarly under the same environmental conditions with a given scion variety.

To obtain such uniform rootstocks, experimentation has been directed toward vegetative propagation, through cuttings or layers of known types of stocks and the comparison of the results produced by stocks thus obtained with the results obtained when the more or less variable seedlings from the same type are used. This work, mainly introduced and stimulated through the investigations of Hatton and his coworkers (1917 and later) on the vegetative propagation of deciduous fruit stocks, has been taken up recently by many American experiment stations.

In the studies of citrus rootstocks which are being conducted by the writer, it was soon recognized that apogamy was likely to exercise an important rôle, as it was known that several of the stocks commonly used exhibited a high degree of apogamic development. The early studies of Webber (1900 and 1900a) and the more recent investigations of Frost (1926) and of Toxopeus (1930) have indicated that the variation in the percentage of apogamic embryos in some of the *Citrus* species and varieties commonly used as stocks is approximately as follows: sweet orange (*C. sinensis*), from 40 to 95 per cent; sour orange (*C. aurantium*), 75 to 85 per cent;<sup>8</sup> grapefruit (*C. maxima*), 60 to 95 per cent; mandarin orange (*C. nobilis*), 10 to 100 per cent; lemon (*C. limonium*), 10 to 96 per cent; citron (*C. medica*), 40 to 50 per cent; and Trifoliate orange (*Poncirus trifoliata*), 72 per cent. This wide variation in the percentage of apogamy shown by different species, and by different varieties or races of the same species it would seem must be of significance in the production of uniform progeny, and thus in the adaptability of the different sorts for use as stocks.

A factor of exceptional interest in this connection is the high percentage of apogamy exhibited by some  $F_1$  hybrids of radically distinct species, and the fact that such hybrids frequently are exceptionally vigorous and likely to possess value as stocks. As an illustration the Trifoliate orange crossed with the sweet orange gave rise to the group

<sup>8</sup> The percentage of apogamy given for the sour orange is based on the count of variants observed in seedling progenies, and not on counts of recognizable hybrids produced through the use of protected flowers crossed with carefully chosen male parents, as is the case for the other estimates given.



of  $F_1$  hybrids which have been designated "citranges" (Webber and Swingle, 1904). Some of these have already attracted attention as desirable stock types. Progenies of several hundred plants of each of several of these hybrid varieties, namely, Savage, Cunningham, Morton, Coleman, and Rusk, have been grown in connection with the writer's experiments and found on careful examination of 3-year-old seedlings to have reproduced apparently true to the variety type ( $F_1$  hybrid) in all cases. The seedlings of these varieties, therefore, are to be considered as approximately 100 per cent apogamic. However, attention should be called to the fact that in the writer's experiments, one of these citranges (the Sanford) apparently develops few if any apogamic embryos and has been found to break up into many types in the  $F_2$  generation.

A high percentage of apogamy is also exhibited by the Sampson tangelo (grapefruit ♀ X tangerine ♂), where the  $F_2$  seedlings produce trees of the same character as the original  $F_1$  hybrid. Twenty-eight  $F_2$  seedlings of the Sampson tangelo, chosen merely as the most vigorous among a progeny of approximately 100 plants, were planted at an age of about 2 years in the variety orchard of the Citrus Experiment Station in 1917. They are now about 16 years old and are adjacent to several budded trees of the  $F_1$  hybrid variety, thus affording opportunity for an easy comparison of their characters with those of the mother type. The  $F_2$  seedlings are remarkable for the uniformity they exhibit among themselves in size, branch, foliage, and fruit characters, and for their vigorous growth. They can be distinguished from the  $F_1$  budded trees only by their more upright growth, which is a character almost invariably exhibited by seedlings as distinct from budded trees.

The examination of a population of over 200 Sampson tangelo 2-year-old  $F_2$  seedlings also showed only typical foliage characters of the variety ( $F_1$  hybrid characters). Thus it is clear that the seedlings of the Sampson tangelo are approximately 100 per cent apogamic under ordinary conditions.

From the high percentage of apogamy occurring in such a wide range of *Citrus* species and varieties, it seems clear that the obtaining of satisfactory stock types that produce seedlings of uniform genetic constitution should present little difficulty, although in most cases a small number of sexually produced embryos develop seedlings.

## NATURE AND INFLUENCE OF VARIATIONS

It has been found that among the progenies of all species and varieties not completely apogamic, there occurs a small proportion of seedlings that differ from the prevailing type of the progeny in character of branching, foliage, and fruit. Most commonly these variants, which are described in the early part of this section, page 7, are comparatively small in size, though some are nearly normal, and it has been shown in experiments described later in this paper that almost invariably they produce some degree of dwarfing in the scions grown on them.

The evidence available indicates that these variants apparently are seedlings produced from the normal (sexual) embryos. It may be that some of these variants come from apogamic embryos and are produced by gene mutation, or by chromosome aberration in the somatic tissue. It is probable, however, that the great majority of them are to be considered as coming from the sexual embryos. Of this majority, an occasional variant seems to be a hybrid produced by cross-fertilization, and mutation may occasionally be concerned, but segregation following self-fertilization seems to be the most probable explanation in most cases. The very general interfertility of *Citrus* species and varieties favors wide natural crossing, and the abundant production of apogamic embryos favors the long persistence of heterozygosis produced by crossing and the gradual increase in the number of heterozygous factors by the accumulation of recessive mutations. The lack of vigor of most of the variants suggests the presence of recessive genes.

Frost (1926, p. 388) states "Selfing probably produces, as a rule, fewer and weaker viable sexual progeny than does crossing. . . . Probably most of the undesirable variant types among nursery seedlings are produced by fertilization. . . . From this point of view, clons which produce seed with fairly numerous embryos are likely to give better results (for stocks) than clons usually with monembryonic seeds. The suitability of the Florida Rough lemon for use as a stock, plainly depends partially on the fact that it is highly polyembryonic and therefore unlike the Lisbon lemon, reproduces mainly by apogamy when selfed."

The elimination of the variants from a batch of nursery seedlings before they are budded (as will be shown later) is apparently the most important selection that can be made in the nursery. The seed-

lings remaining after such an elimination, if from the same mother tree or clon, can be safely considered to be chiefly of apogamic origin and of nearly uniform genetic constitution. Such seedlings should possess the same degree of congeniality with the scions of any given fruit variety worked on them by budding or grafting; and the reactions produced under a given set of conditions can be determined with sufficient accuracy so that apparently the same result can be expected to follow whenever the same combinations are used under the same set of conditions.

Probably no such certainty of results could ever be obtained by the use of variable seedlings of differing genetic constitution, such as those obtained from cross-pollinated plants that develop seeds in the normal way from the fertilized egg cells only. Apogamy thus apparently furnishes the citrus nurserymen with a means of obtaining easily from any known good stock type, large batches of seedlings that can be depended upon to be of nearly uniform genetic type and to give a uniform reaction on the scion. This result has apparently been of great value to the industry in the past, although not generally recognized, and is likely to be of even greater importance in the future as more is learned about the conditions and the reactions to be expected.

Seedlings of all citrus species (whether of apogamic origin or developed from egg cells in the normal way) are, of course, subject to the same environmental influences as are other plants, and show the same general classes of variation. Developmental or environmental variations are of course shown by both types of citrus seedlings, and the extent and influence of such variations in seedling progenies will be discussed later. Mendelian or genetic variations are exhibited in seedlings from the sexually produced embryos, but should not show in the apogamic seedlings, which supposedly are of uniform genetic constitution. Mutations are likely to occur among seedlings from either of the two types of embryos, but are not common. In the discussions in this paper they probably would be classed merely as variants and eliminated as such. In the following discussions of populations grown in connection with different experiments, it should be remembered that the seedlings continually referred to as variants are those which probably have come from the sexual embryos in most cases. The different characters exhibited by them are probably to be considered as Mendelian or genetic variations. Some of these variants may be mutations, but this could not be determined without extended investigations.

After the exclusion of the variants, the remaining population (referred to as "entire population without variants") is to be considered as seedlings from apogamic embryos that possess the same genetic constitution as the mother parents from which the seed came. As the seeds in these experiments were not taken from single trees, there may be some variation in the genetic constitution of these populations even after all variants are eliminated.

### TESTS OF LARGE, MEDIUM, AND SMALL NURSERY TREES ON SWEET-ORANGE STOCKS<sup>9</sup>

In a nursery planted in 1914, and intended to supply some 5,000 trees to be used in starting a fertilizer experiment at the Citrus Experiment Station of the University of California, greater than ordinary uniformity among the trees was desired, and much care was thus taken in the growing and handling of the nursery (see Batchelor, Parker, and McBride, 1928). The seed used in growing the rootstocks was taken from four old seedling trees in the grove of R. S. Thompson at Highlands, California, and these four trees were from seed taken from sweet oranges from Tahiti and planted in 1886.

When the seed bed was dug the small seedlings were discarded to the extent of about 10 per cent of the whole number. The others were planted in the nursery, handled as uniformly as possible, and, with the exception of small, deformed, or apparently "off-type" seedlings, were budded at the same time with buds from highly selected trees. When the budlings had reached the age to be transplanted into the orchard they presented the appearance of an exceptionally fine block of very uniform nursery trees; and yet, when the selection of trees was made to plant the experiment in question, it was found that the sizes of the scions, 3 to 4 inches above the bud union, varied from 0.85 cm to 3.00 cm or more in diameter.<sup>10</sup>

In order to obtain some clue as to what would have been the result if all sizes of the nursery trees had been used without selection, a comparative trial was planted with 18 large, 18 medium, and 18 small trees of each of three varieties: Washington Navel orange, Valencia orange, and Marsh grapefruit, all on sweet-orange stocks, a total of

<sup>9</sup> For earlier reports on this experiment, with photographs showing the comparative size of the trees, etc., see Webber (1919, 1920, and 1920a).

<sup>10</sup> The selected budlings from this nursery that were planted in the fertilizer experiment referred to have furnished some interesting data that is summarized on page 54 of this paper.

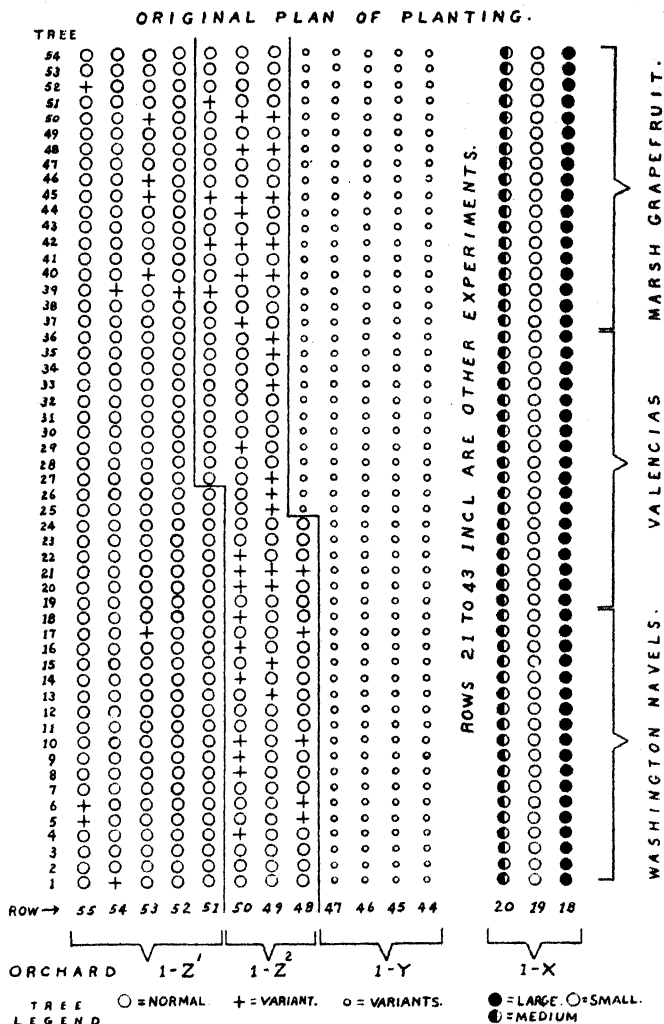


Fig. 2. Outline plan of experimental orchards, the results from which are discussed in this paper. All in field 1, blocks A, B, and C, Citrus Experiment Station, Riverside, California.

1-X: Rows 18-20. Tests of large, medium, and small budlings of Washington Navel and Valencia oranges and Marsh grapefruit. All on sweet-orange stocks. Planted in 1917.

1-Y: Rows 44-47 and part of 48. Orchard of variant types used as stocks in orchard 1-Z. Planted in 1922.

1-Z: Rows 48-55. Orchard of Washington Navel orange budded on known types of sour-orange seedlings. Planted in 1922.

1-Z<sup>1</sup>: On first-grade seedling stocks chosen at seed bed as large.

1-Z<sup>2</sup>: On second-grade seedling stocks chosen at seed bed as small.

162 trees. The large, small, and medium trees of each variety were placed in adjacent rows to facilitate visual comparison (see planting plan, fig. 2, orchard 1-X, rows 18, 19, and 20). The average size of the trees in each of the large, medium and small groups of each variety at the time of planting, as indicated by area of trunk cross section, is shown in column 2 of table 1.

The trees were planted 10 feet apart, in rows 24 feet apart, and by 1928 (when they were 11 years old) those in the row planted with large seedlings were crowding each other severely. Every alternate tree was removed in the spring of 1929, to provide for the normal growth of those remaining. Therefore the summary of the results presented here is limited to the period from the spring of 1917 up to the spring of 1928, a total of 11 years. In judging the results it should be borne in mind that during the last 2 or 3 years of this period, the trees (particularly those in the rows planted with large nursery stock) had doubtless been somewhat injured and reduced in size and yield by the crowding.

In this study of the effect of size of budlings (nursery trees) on the later size and yield of the same trees in the orchard, the data used are derived from measurements of trunk area, top volume, and total yields of each tree during 6 years. The average yields were derived from the weight of fruit, in pounds, produced annually during the last 6 years of the period (crops of 1922-23 to 1927-28 inclusive), thus beginning after the trees had reached bearing age (first measurement made in sixth year). The averages of these measurements for each plot are presented in table 1.

TABLE 1  
SUMMARY OF RESULTS OBTAINED WITH LARGE, MEDIUM, AND SMALL NURSERY  
TREES AS SHOWN BY AVERAGES

Variety	Tree size	Average trunk area in 1917	Average trunk area in 1928	Average top volume in 1928	Average total yield per tree, 6-year period
	1	2	3	4	5
		sq. cm.	sq. cm.	cu. ft.	lbs.
Washington Navel orange	Large.....	5.052	119.43	620.61	419.5
	Medium.....	2.241	110.44	508.27	416.0
	Small.....	1.355	95.97	440.29	350.3
Valencia orange	Large.....	5.275	161.40	858.00	349.17
	Medium.....	2.055	138.15	664.83	320.78
	Small.....	1.057	140.60	712.93	425.69
Marsh grapefruit	Large.....	7.404	154.43	710.93	783.64
	Medium.....	2.750	124.94	557.44	693.97
	Small.....	0.871	120.20	434.33	576.73

It will be seen from an examination of the data given in table 1 that, during the 11-year period up to 1928 in which the trees were growing in the grove, in general the large trees remained large; the medium trees, medium; and the small trees, small (fig. 3); and also that the size of the yield corresponds to the size of the tree. The one exception among the 9 plots is found in the Valencias, where the 18 small trees are larger in size than the medium trees and have given a better average yield than either the medium or large trees.



Fig. 3. Marsh grapefruit trees, 13 years old, on sweet-orange stocks showing the influence of budling selection on tree size. Row on left grown from selected large budlings; row on right grown from small budlings. Photographed January, 1930.

In order to obtain a more exact expression of the size and yield relations of these trees, the whole population (large, medium, and small) of each variety was considered together. The coefficients of correlation were determined for trunk area of original nursery tree in 1917 with 1928 trunk area, with 1928 top volume, and with average 6-year yields. These data are given in table 2.

It will be seen from an examination of these data for the combined populations that significant correlations exist in all cases where size is considered. If area of trunk cross section of the budlings is compared with similar measurements of the corresponding 11-year-old orchard trees, the coefficient for the Washington Navel is  $+0.394 \pm 0.082$ ; for the Valencia,  $+0.362 \pm 0.082$ ; and for the Marsh,  $+0.460$

$\pm 0.077$ . These correlations are moderately large and in each case they are more than four times larger than their respective probable errors and may thus be considered significant.

The same is true for all varieties when area of trunk cross section of budlings is compared with top volume of orchard trees, the correlations being for Washington Navel,  $+0.452 \pm 0.072$ ; for Valencia,  $+0.390 \pm 0.081$ ; and for Marsh,  $+0.582 \pm 0.064$ . These are slightly larger correlations than those obtained when trunk area of budling was compared with trunk area of orchard trees, and it will be noticed that in each case they are about the same relative amount larger.

TABLE 2

CORRELATION OF SIZE OF BUDLINGS WITH SIZE AND YIELD OF ORCHARD TREES (ALL ON SWEET-ORANGE STOCKS)

Variety	Number in population	Data correlated	Coefficient of correlation
Washington Navel orange	{ 49	Trunk area 1917 to trunk area 1928 .....	$+0.394 \pm 0.082$
	{ 49	Trunk area 1917 to top volume 1928 .....	$+0.452 \pm 0.072$
	{ 49	Trunk area 1917 to total 6-year yield .....	$+0.170 \pm 0.092$
Valencia orange	{ 52	Trunk area 1917 to trunk area 1928 .....	$+0.362 \pm 0.082$
	{ 52	Trunk area 1917 to top volume 1928 .....	$+0.390 \pm 0.081$
	{ 52	Trunk area 1917 to total 6-year yield .....	$-0.141 \pm 0.093$
Marsh grapefruit	{ 47	Trunk area 1917 to trunk area 1928 .....	$+0.460 \pm 0.077$
	{ 47	Trunk area 1917 to top volume 1928 .....	$+0.582 \pm 0.064$
	{ 47	Trunk area 1917 to total 6-year yield .....	$+0.410 \pm 0.085$

The difficulty of obtaining trustworthy comparative results from plot experiments, unless the plots are replicated several times, is well recognized. It is, nevertheless, considered particularly significant that the trees in each of these 9 plots should have continued for a period of 11 years to exhibit approximately the same relative size that they did in the beginning of the experiment.

Trunk measurements of these trees were taken several times during the 11-year period of the experiment, and it is of interest to note that the average size of each group has continued throughout in about the same relative position. This is shown graphically for the Washington Navels and Valencias in figure 4. The average size of the trees remaining in 1931 after the thinning of the orchard is included in this graph.

It will be seen from an examination of the graph that the lines representing the relative increase in size of the trees of each group rise rapidly and gradually approach nearer together as the trees grow



older. The large group of trees in each case still retains its superiority, but it is indicated that in a longer-continued period of time the relative increase of the large and small trees in each varietal group may become equal.

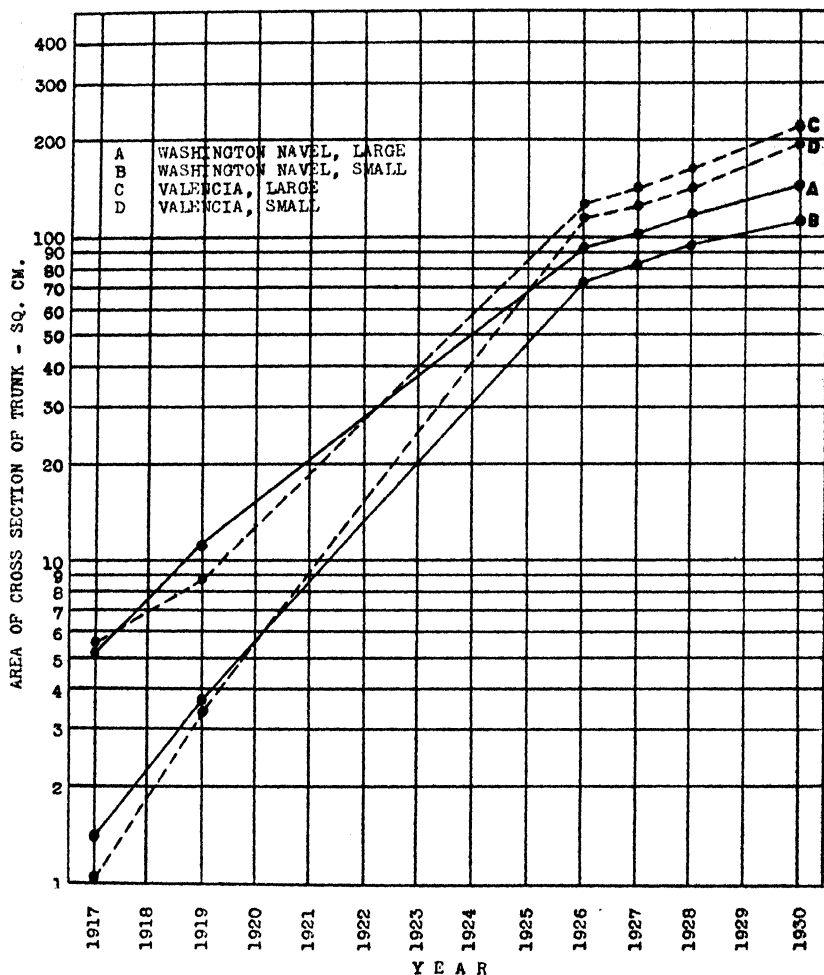


Fig. 4. The relation of relative increase in size at various intervals of time, for the large and small trees of Washington Navel and Valencia oranges as shown by area of trunk cross section. Solid lines, Washington Navel: A, large; B, small. Dash lines, Valencia: C, large; D, small.

In the case of the yields, the results from these plots show some complications. In Marsh grapefruit, the correlation of 1917 budling trunk area with average total 6-year yield, which is  $+0.410 \pm 0.085$ ,

would be considered as significant. However, the same correlation with the Washington Navel is only  $+0.170 \pm 0.092$ , which is not twice as large as the probable error and could scarcely be considered as significant, and with Valencia is  $-0.141 \pm 0.093$ , a negative correlation which would certainly not be considered significant.

It is unfortunate that in this experiment the plots of different-sized trees were not replicated several times with each variety to furnish a direct check on any outstanding difference, such as is shown by this one plot of small budlings of Valencia. The other 8 plots may be considered in a sense as check plots since they have all retained their same relative rank in all of the three characters measured.

In the studies of Parker and Batchelor (1932) on the early yields during the first 10 years of the different plots of Washington Navels that were to be used in the fertilizer experiments of this Station, it was found that adjacent plots frequently varied greatly in yield and maintained the same rank uniformly throughout the first 10 years (6 fruiting years) during which time all plots were treated uniformly. Such differences were assumed to be due to soil variations or possibly to some extent to differences in methods of planting used by different planting crews, although all trees were handled as nearly alike as possible. These fertilizer experiments are on the same type of soil in the same field as the writer's experiments and thus it appears probable that certain limited patches of soil which seem to be uniform vary sufficiently to be responsible for these variations in plot yields.

While it seems probable that soil variation may be responsible for the exceptional result produced by the Valencia plot of small trees, no outstanding difference in the soil can be observed. Furthermore, the trees in this experiment so far as can be traced in the records or from the memory of those who did the work, were all planted by the same crew. The reason why this one plot of Valencias forms an exception cannot be clearly explained at the present time.

It is probable that during the last 2 or 3 years of the entire 12 years of this experiment, the plots of large budlings were slowed up, in growth and yield, more than those planted with medium or small budlings, due to the crowding, which was most noticeable in the row planted with large budlings. At least it is certain that whatever effect resulted from the crowding would have been favorable to the plots of small trees.

When all factors are considered, however, the fact remains that the evidence from the records of comparative area of trunk, volume

of top, and even of yield during the period of the experiment, in general indicates the continued superiority of the selected large budlings over the small ones.

If this is the case, which was not assumed when the experiment was started, it is important to determine, if possible, the factor or factors fundamentally responsible for this variation in budlings in order that the largest possible proportion of the superior budlings may be grown, and means found of eliminating the inferior ones.

In the propagation of the trees used in this experiment, buds were taken from carefully selected trees of known performance record, except in the case of the Valencias, the buds for which were taken from good trees true to type, but the performance records of which were not known. It is assumed, however, that the variations in size of budlings were probably not due to differences in the buds used.

When the budlings were dug in order to transplant them into the orchard, they were taken up "bare root" and the roots carefully examined for possible malformations or diseases that might render them inferior. No individuals were chosen for planting except those that were judged to have normal healthy roots, and thus it is not believed that malformation of the roots or any diseased condition can be considered as responsible for the small trees in this experiment.

The bud unions were also carefully examined and all the plants chosen had apparently healed over promptly and formed normal unions.

Probably the most common causes for ordinary variations in size of nursery trees and plants in general are local variations in the environment under which they are grown, such as richness of soil, texture of soil, moisture supply, etc. If, however, such environmental factors in the nursery were responsible for all of the variations in size among the budlings used in this experiment, it would seem probable that when the trees were removed and planted in the orchard under new environmental conditions, in most cases they would soon have responded to the new conditions and grown out of their original rank of size. It would seem that the very slight handicap in size of the small budlings, if caused only by the nursery environment, would soon be overcome and be unrecognizable in the new environment. Some of the small budlings have indeed produced good-sized trees and some of the large budlings have not retained their original rank in size, but in general, the small have remained small and the large have remained large.

The only other obvious variable is that introduced by the rootstocks, and these were seedlings of good selected sweet-orange trees, but of unknown heritage. They were taken from a bed in which the seedlings had made excellent growth and the smallest seedlings to the extent of about 10 per cent of the total number had been discarded when the seedlings were dug and transplanted to the nursery. Seedlings of many citrus species are known to be more or less variable. Most of the varieties grown are known to be heterozygous for many characters so that they do not reproduce true through the seed. Thus it may seem reasonable to suppose that the seedlings used as stocks at least in many cases, were variable and of hybrid nature for certain characters so that they might be expected to react differently upon the scions grown on them. However, the selection preceding the budding had probably eliminated most of the variants, so that there remained a nearly homogeneous lot of seedlings of apogamic origin.

This experiment served to focus attention on rootstocks as a probable cause of variation in the size of orchard trees. It also pointed out the necessity for a more careful study of variations occurring among seedlings, and the influence of such variations on the size and general character of the trees of varieties worked on them.

## REACTION ON SCIONS CAUSED BY DIFFERENT-SIZED ROOTSTOCK SEEDLINGS

### PLAN OF EXPERIMENT

In order to obtain definite evidence as to the reaction on scions caused by rootstock seedlings of different size and type, it was necessary to make actual trials, and such an experiment was started in 1919.<sup>11</sup>

<sup>11</sup> This experiment, which was planned and started by the writer, was carried out during the important period from 1921 to 1926 by Dr. J. T. Barrett, then Acting Director of the Citrus Experiment Station. Dr. Barrett thus deserves much credit for the results obtained, but is not to be held responsible for the interpretation of the results and the conclusions reached as stated in this paper. Figures 6 to 12 are from photographs taken by Dr. Barrett. The writer again took charge of the experiments in 1926.

A preliminary report on this experiment, prepared by the writer in cooperation with Dr. Barrett, was presented before the Ninth International Horticultural Congress held in London, England, August 8 to 15, 1930, and was published in the proceedings of the Congress (Webber and Barrett, 1930). Some of the discussion of this experiment given in the present paper is taken with little change from that report, but the data are given here in more detail, and considerable new material has been added.

Sour-orange seedlings were taken from an ordinary seed bed grown at the Citrus Experiment Station in the spring of 1919 when they were 1 year old, and the very smallest seedlings were discarded, to the extent of about 10 per cent of the total population. Such very small seedlings are commonly discarded by nurserymen when a seed bed is dug, because they are too small to transplant well and usually fail to grow after transplanting. The remaining seedlings, ranging in height from about 4 to 12 inches, were then examined and segregated by sight judgment into two lots by size (large and small), and these lots were planted separately in the nursery. These will be designated in the further discussion as first and second grade, or merely as firsts and seconds.

After they had grown one year in the nursery, the lot of first-grade large seedlings, 301 in number, ranged in height from 5 to 30 inches with an average of 18.19 inches, while the lot of second-grade small seedlings, numbering 228, ranged from 2.5 to 20 inches in height with an average of 9.07 inches (Webber, 1920*a*, p. 292). A considerable number of the small and weak seedlings, especially among the seconds, had died before measurements were taken.

These seedlings were given permanent individual numbers in the spring of 1921 and were carefully studied as to type and size (diameter and height), after which they were budded with buds taken from one carefully selected Washington Navel tree.<sup>12</sup> In the spring of 1922 these budded trees were planted in a permanent experimental orchard, in the same order that they occupied in the nursery, and this orchard is here designated as orchard 1-Z (see planting plan, fig. 2).

At the time when the tops of the above seedlings were cut off to force the Navel buds into growth according to the common nursery practice, bud sticks were taken from the tops of each variant seedling, and two trees were budded with each, one on Rough-lemon stock, and one on sour-orange stock. By this means, two trees representing the type of each of the variant sour-orange seedlings, which were budded to Washington Navels, were retained as budded trees in order to permit the study of their mature tree characters. Each was propagated on two different rootstocks in order that judgment might be formed

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<sup>12</sup> The buds used for this purpose were from tree 3-14-27, located on the Vivienda Ranch of the National Orange Company at Highgrove, California, which was the tree ranking No. 1 in the block of performance-record trees studied and described by Shamel, Scott, and Pomeroy (1918). The Station is greatly indebted to the National Orange Company and to A. D. Shamel and his coworkers for the privilege of using buds from this tree.

as to whether the rootstock influenced in any material degree the type of the variant.

The nursery trees propagated from these variant seedlings were also planted in an experimental orchard adjoining orchard 1-Z, and is here referred to as orchard 1-Y (see fig. 2).

These two orchards, No. 1-Z and No. 1-Y, were 8 years old in 1929 when this study of the data was started, and had been fruiting for several years. This experiment furnishes a case where the results from growing uniform buds on known types of seedlings, normal and variant, can be studied and the reactions observed (orchard 1-Z); and if a tree on a seedling that was classed as a variant shows any peculiar reaction in orchard 1-Z, the type of that particular variant can be studied in the orchard of variant types (orchard 1-Y).

In the propagation of the budlings for this experiment, 289 first-grade seedlings were budded, and among these 16, or 5.5 per cent, were distinguished as variants. Two hundred and ten second-grade seedlings were budded, among which 69, or 32.9 per cent, were distinguished as variants. The variants were so classed not only because of their size but also because of morphological differences.

There was some loss from buds that failed to grow and a considerable number of trees have died in the course of the experiment, particularly those on variant seedling roots, so that the population remaining for study and comparison has been reduced to 241 of the large first-grade stock seedlings, among which there are 10 that were classed as variants; and 148 of the small second-grade stock seedlings, among which there are 33 that were classed as variants. The much greater frequency of variant types among the smaller-sized or second-grade seedlings as separated at the time they were dug from the seed bed, is very noteworthy.

The original size of the rootstock seedlings at the time of budding as shown by the area of cross section of trunk 4 inches above the ground, has been compared with the size of scion trunk when 1 year old in the nursery and when 8 years old as indicated by area of trunk cross section. Studies and comparisons have also been made between the size of the scion trunk when 1 year old (as a nursery tree) and the size the same trees have attained after 8 years' growth in the orchard as shown by area of trunk cross section, and with the yield as shown by the total yields of each tree up to 1930. Use has been made of the ordinary statistical constants such as the mean, standard deviation, coefficient of variability, and coefficient of corre-

lation, in determining the growth relation and yield for the entire population and for various groupings of the population in different cases.

#### PERMANENCE OF VARIANT TYPES

In the consideration of the data derived from this experiment it is desirable first to know whether the variants noted among the population of seedlings used as stocks have continued to show differential characters indicating genetic differences.

Orchard 1-Y, where each of these variants was propagated upon two different rootstocks, presented after 8 years of growth, a medley of types that would be difficult to exceed if one were to bring together all of the most diverse species of *Citrus* (fig. 5). A very few of those chosen as variants approach closely to the normal type of the sour orange and are of approximately normal size for their age (fig. 15, tree 46-32). Many remain veritable dwarfs, being only  $2\frac{1}{2}$  to 3 feet high (fig. 9) at an age when normal sour-orange trees should have reached 10 feet or higher. All sizes between these two extremes are exhibited by the different variants (figs. 11, 13, and 15). Other characters apparently exhibit fully as great a range of variation as does size, and one finds extremes of coarse and slender branching, open and dense foliage, long and short leaves, broad and narrow leaves, broadly winged and nearly wingless petioles, large and small fruits, light yellow and orange-red fruits, well-developed glands and strong odor or atrophied glands and odorless. Some of the trees with shapely tops of finely branched stems and with dense foliage of slender pointed leaves would scarcely be recognized as citrus trees. The great majority of them exhibit some degree of sterility and some have as yet shown no indication of flower development.

It is important to note that the two different rootstocks, Rough lemon and sour orange, used in the propagation of each of these variants, has had little or no influence on the type of the variant as indicated by the visible characters. The trees of each type on the two stocks are growing side by side and invariably show the same characteristics. The trees on Rough-lemon stock, in general, are somewhat larger than those of the same type on sour stock, though there are some exceptions. Invariably, however, the distinctive character of the type remains unchanged. If it is a dwarf type both trees are relatively dwarfed, or if it is a dense-foliage type with long narrow leaves, both trees show the same characters without reference to the stock. The Rough lemon is a very vigorous-growing stock,

very distinct from the sour orange, and it is rather surprising that these widely variant types of the sour orange when budded onto it show such slight differences, or stionic effects, in comparison with the same trees budded on a standard type of the sour orange where the affinity is certainly much closer (figs. 5 and 9). The only differences observable without an exhaustive study are limited to size characters, indicating that the stock is to be considered here merely as furnishing a different environment for the growth of the scion, which it affects only in such characters as are modified directly by the environment. The results here are entirely confirmatory of those obtained in the experiment outlined in a preceding section.



Fig. 5. Variant sour-orange types used as stocks (orchard 1-Y, row 46); two trees of each variant, the one on the right of each couple being propagated on Rough-lemon stock and that on the left on sour-orange stock. Note that the character of each variant remains unchanged by stock influence. The variant seedling from which couple *A* was propagated is the stock of Washington Navel 49-25; couple *B* is the stock of 49-26; and couple *C* the stock of 49-27; see figure 6. All 8 years old.

#### INFLUENCE OF VARIANT SEEDLINGS IN PRODUCING DWARFED ORCHARD TREES

Greatest interest centers in orchard 1-Z where the variant seedlings represented in orchard 1-Y are used as rootstocks, together with the normal seedlings in the same population. As the buds from one single selected Washington Navel tree were used in propagating



these trees, they should be exceptionally uniform. Such is the case with the lot of large seedlings, but the small seedlings have given a highly variable lot of trees as judged by size (figs. 6 and 7). A tabulation of the results shows that almost every seedling that was classed as a variant has, when budded, produced an orchard tree exhibiting some degree of dwarfing and in the majority of cases very marked dwarfing (fig. 8, tree 49-15; fig. 10, tree 50-14; and fig. 14, tree 49-25). This is the case even where the variant itself is nearly



Fig. 6. Washington Navel orange on sour-orange stocks: tree 49-24 on normal stock; tree 49-25 on variant seedling stock (see fig. 5A); tree 49-26 on variant seedling stock (see fig. 5B); tree 49-27 on variant seedling stock (see fig. 5C). Note the variation in size due to stock. All 8 years old, orchard 1-Z<sup>2</sup>, row 49.

normal in size, indicating only a slight depression of the normal growth rate as shown by its growth in orchard 1-Y (compare figs. 10 and 11).

As the largest proportion of the variant types was in the lot of small seedlings, the portion of orchard 1-Z planted with second-grade small stock seedlings (1-Z<sup>2</sup>) shows a higher proportion of these dwarfed trees, while the portion of orchard 1-Z planted with large stock seedlings (1-Z<sup>1</sup>) shows only a very small number of such trees. For an understanding of this section compare figures 6 to 15 inclusive.

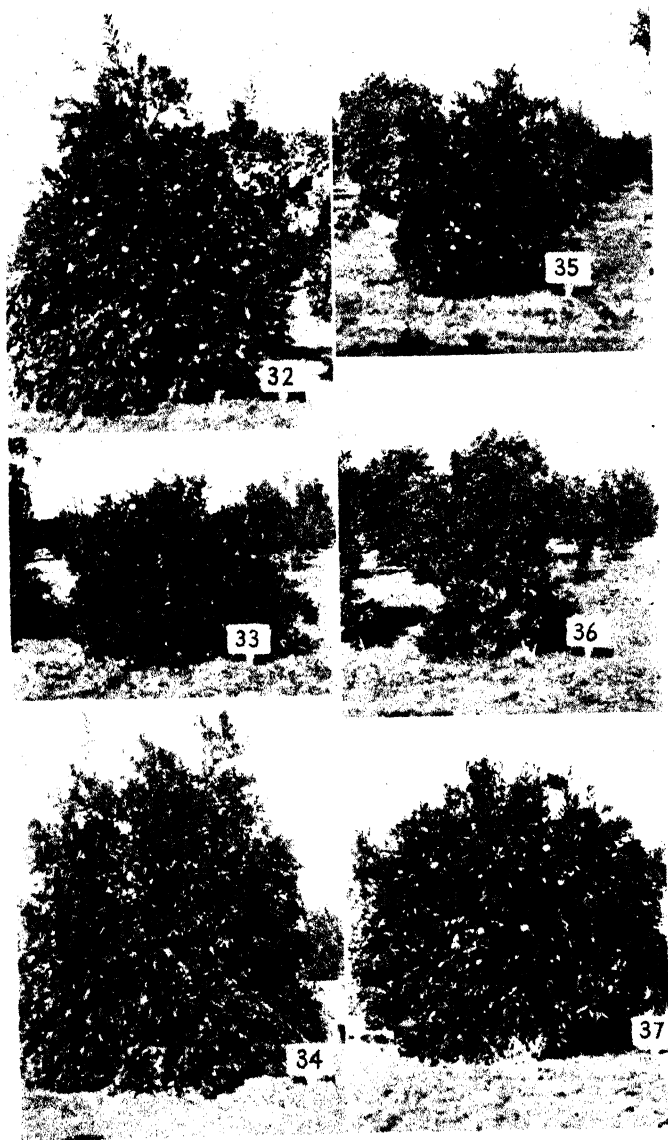


Fig. 7. Six consecutive trees in row 49 of Washington Navels on second-grade seedlings of sour orange in orchard 1-Z. Trees 32, 34, and 37 are normal-sized trees on seedlings of normal type. Trees 33, 35, and 36 are dwarfed trees on seedlings of variant type. All trees 8 years old. Note the different degrees of dwarfing.



Fig. 8 Washington Navel orange on sour orange stocks: tree 49-15, a dwarfed tree on a variant seedling stock (see fig 9, trees 46-12 and 46-13); tree 49-14, a normal sized tree on a normal sour orange seedling. Trees 7 years old.



Fig. 9. Variant sour orange types used elsewhere as stocks: trees 46-12 and 46-13 were propagated from a small dwarf seedling which is the stock of Washington Navel 49-15 in figure 8. Note also the variation in the other variant sour orange types in the background. The couple immediately in rear of tree 46-12 propagated from one variant seedling, show clearly that they are the same type, although the one on the right, which is on Rough lemon stock, is slightly larger than the other, which is on a normal sour orange stock. The couple immediately in rear of tree 46-13 are propagated from still another variant seedling and are intermediate in size between the other two types shown in this photograph. Trees 7 years old.



Fig. 10. Washington Navel on sour-orange stocks: tree 50-14, a dwarf tree on a variant seedling stock (see fig. 11, trees 47-23 and 47-24); tree 50-13, a standard-sized tree on a normal sour-orange seedling. Trees 7 years old.



Fig. 11. Variant sour-orange types used elsewhere as stocks: tree 47-23 on Rough-lemon stock and 47-24 on sour-orange stock, both propagated from the same variant seedling used as the stock of Washington Navel 50-14 in figure 10. This is an instance of a nearly normal-sized variant which as a stock dwarfs the scion. Trees 7 years old.



Fig. 12. Washington Navel on sour-orange stocks: tree 48-10, a slightly dwarfed tree on a variant seedling stock (see fig. 13, trees 45-13 and 45-14); tree 48-9, a standard-sized tree on a normal sour-orange seedling. This is a case of a vigorous-growing variant that produces only a slight dwarfing effect. Trees 7 years old.



Fig. 13. Variant sour-orange types used elsewhere as stocks: tree 45-13 on Rough-lemon stock and tree 45-14 on normal sour-orange stock, both propagated from the same variant seedling used as the stock of Washington Navel tree 48-10 in figure 12. Note the same general type of these two trees although on widely different stocks. Trees 7 years old.



Fig. 14. Washington Navel on sour-orange stocks: tree 49-25, a severely dwarfed tree on a variant seedling stock (see fig. 15, tree 46-30); tree 49-26, a slightly dwarfed tree on a large vigorous growing variant stock (see fig. 15, tree 46-32). Trees 7 years old.



Fig. 15. Variant sour-orange types used elsewhere as stocks: tree 46-30, on Rough-lemon stock, a medium-sized variant propagated from seedling used as stock of tree 49-25 in figure 14; tree 46-32, on Rough-lemon stock, a large variant propagated from seedling used as stock of tree 49-26, in figure 14. Trees 7 years old.

# INFLUENCE OF SIZE OF ROOTSTOCK SEEDLINGS ON SIZE AND YIELD OF ORCHARD TREES

The next point to be considered in the analysis of the data on this experiment is the effect which the size of the original rootstock seedlings in orchard 1-Z had on the size of the scions grown on them. In table 3 the statistical constants for this entire population of trees are given at three periods, namely, for the size of the rootstock seedlings in 1921, size of scion in 1922, and size of scion in 1929, with correlations between the two latter and the former.

TABLE 3

COMPARISON OF SIZE OF ROOTSTOCK SEEDLINGS AT TIME OF BUDDING WITH SIZE OF  
BUDLINGS AT 1 YEAR AND 8 YEARS

(Area of cross section of seedling trunk in 1921 with area of cross section of scion trunk in 1922 and 1929, respectively; population 397\*)

Constants	Area of trunk		
	Stock seedlings, 1921 (4 in. above soil)	Scion, 1922 (2 in. above bud union)	Scion, 1929 (6 in. above bud union)
Age of population.....	3 yrs. from seed	1-yr. budlings	8-yr. trees
Mean, in sq. cm.....	3 55±0 053	2 46 ± 0 032	76.08 ±0.671
Standard deviation in sq. cm.....	1 53±0 037	0.93 ± 0 022	19 55 ±0 473
Coefficient of variability, in per cent.....	43.23±1.228	37.72 ± 1 035	25.70 ±0.662
Coefficient of correlation with area in 1921.....		+0.736±0 016	+0.437±0 028

\* The use of a population of 387 trees here, instead of 389 as in certain other tables, is due to the loss of the measurements of two seedlings in the nursery.

It will be seen from an examination of the data presented in table 3 that the coefficient of variability in size of the 1-year-old budlings is 37.72 per cent, slightly less than that exhibited by the stock seedlings, which is 43.23 per cent. The variability of the trees at 8 years of age is still less, being only 25.70 per cent. In view of the fact that the budlings were all propagated with buds from one selected tree, and therefore presumably all have about the same inherent growth rate, it is rather to be expected that they would show less variation than the variable stock seedlings. Again in the young life of the budlings, variation in the time that the bud starts, owing to minor incidents of variation in method, is likely to show pretty clearly in the size of the top. This variation in budling size is usually more clearly marked during the first year and gradually becomes less evident in succeeding years.

The very marked relation of the size of the stock seedlings in 1921 at the time of budding, to the size of the 1-year-old budlings propagated on them, while still in the nursery under the same environmental conditions, is clearly shown by the high coefficient of correlation,  $+0.736 \pm 0.016$ . As the trees become older, after transplanting to the orchard, this degree of correlation is lessened and the scion trunk size of the 8-year-old orchard trees compared with stock trunk size at the time of budding gives a correlation coefficient of only  $+0.437 \pm 0.028$ . This, however, is a sufficiently large and significant correlation to indicate the general tendency of the large seedlings to produce large trees.

The population of 387 trees in table 3 includes 41 trees which are known to be variants and to produce dwarfed orchard trees. These variants must greatly influence the correlations exhibited when the total population is considered, and it is important to know what occurs when the known variants are excluded from the population and only trees used that are supposed to be of normal type.

In table 4 corresponding data are given for the population exclusive of variants.

TABLE 4

RELATION OF SIZE OF STOCK SEEDLINGS TO SIZE OF BUDLINGS AND ORCHARD TREES, WHEN VARIANTS ARE EXCLUDED

(Area of cross section of seedling trunk compared with areas of cross section of scion trunk at different ages; population of 346)

Constants	Area of trunk				
	Stock seedlings, 1921 (4 in. above soil)	Scion, 1922 (2 in. above bud union)	Scion, 1924 (4 in. above bud union)	Scion, 1927 (4 in. above bud union)	Scion, 1929 (6 in. above bud union)
Age of population	3 years from seed	1-year-old budlings	2-year orchard trees	6-year orchard trees	8-year orchard trees
Mean in sq. cm.	3.87 $\pm$ 0.046	2.69 $\pm$ 0.025	9.73 $\pm$ 0.065	53.54 $\pm$ 0.309	81.05 $\pm$ 0.422
Standard deviation in sq. cm.	1.28 $\pm$ 0.033	0.68 $\pm$ 0.018	1.79 $\pm$ 0.046	8.52 $\pm$ 0.218	11.63 $\pm$ 0.298
Coefficient of variability, in per cent	33.05 $\pm$ 0.934	25.43 $\pm$ 0.692	18.43 $\pm$ 0.488	15.92 $\pm$ 0.418	14.35 $\pm$ 0.375
Coefficient of correlation with 1921 seedling area		+0.549 $\pm$ 0.026	+0.125 $\pm$ 0.036	+0.010 $\pm$ 0.036	-0.021 $\pm$ 0.037

A number of very significant facts are brought out by the data presented in this table. The coefficient of variability, which, for the seedlings at 3 years of age, just before they were budded, was 33.05  $\pm$  0.934 per cent, is, for the scions grown on them at 1 year of age, only 25.43  $\pm$  0.692 per cent, and this becomes less each year until



at 8 years of age the variability exhibited is only  $14.35 \pm 0.375$  per cent. This shows clearly the general tendency of the degree of difference in size to be smoothed out as the trees grow older.

In this population, where the known variants are excluded, the correlation in size between the seedlings and the 1-year-old budlings as shown by trunk area was only  $+0.549 \pm 0.026$ , whereas for the entire population with variants included, the coefficient was  $+0.736 \pm 0.016$ . It is thus seen that the variants had a marked influence in increasing the degree of correlation.

The most interesting factor brought out by the data in table 4 is the decreasing correlation between size of trunk area of the seedlings with trunk area of the scions as the trees increase in age. This correlation, which in 1922, when the budlings were 1 year old, was  $+0.549 \pm 0.026$ , had fallen in 1924 to  $+0.125 \pm 0.036$ , in 1927 to  $+0.010 \pm 0.036$ , and in 1929 when the orchard trees were 8 years old to  $-0.021 \pm 0.037$ . The last two of these correlations, one slightly positive and the other slightly negative, seem to indicate clearly that the correlation which existed between the size of the seedlings and the size of the 1-year-old budlings entirely disappears as the trees grow older, and that there is no permanent and sustained relation through the life cycle of those trees of the population that remain after the variants are excluded. This is very significant, if it represents the general conditions in other similar citrus populations.

The above data concern only the size of the stock seedlings with relation to the size of the scions grown upon them. It is of some interest to note that the sizes of the trunks of the stocks in the orchard trees react in almost the same way and degree. The trunk area of the stock seedlings correlated with the trunk area of the stocks of the orchard trees when 8 years of age, for the above population of 387 trees including the variants, was  $+0.398 \pm 0.029$ ; while for the same population exclusive of variants, 346 trees gave a correlation coefficient of  $-0.054 \pm 0.032$ , thus corresponding very closely with the relation shown above in comparing size of seedling with size of scion trunk.

Using the volume of top as a measure of size of the trees in June, 1930, when  $8\frac{1}{2}$  years old, and correlating this with the size of the seedlings as shown by area of trunk, the entire population, exclusive of variants, gave a coefficient of  $-0.012 \pm 0.036$ . This indicates that there is no significant relation.

It is important also to know the relation between the size of the stock seedlings and the yield of the orchard trees grown on them.

Here with the entire population exclusive of variants (346 trees), when area of trunk of stock seedlings is compared with the total 5-year yields of the 8-year-old orchard trees grown on them, there is a correlation of  $+0.135 \pm 0.035$ . Thus there is in this case a low positive correlation.

These results all indicate that in the population remaining after the elimination of the variants, the original seedling size apparently had but little or no influence on the final orchard tree size and yield.

In an earlier section of this paper attention was directed to the very common occurrence of apogamy in citrus reproduction. In the sour-orange seedlings used as stocks in this experiment it is likely that those classed as variants can fairly safely be considered as coming from sexually produced embryos, while those considered of normal type probably come mainly from apogamic embryos. The individuals remaining in the population after the exclusion of the variants, therefore, are to be considered as of apogamic origin and of the same genetic constitution as the mother parent or parents.

In this limited population with the variants excluded, there is still the possibility of some genetic variation even though all are of apogamic origin. No record was made of the particular tree or trees from which the seed was taken, but since the seed was gathered in the orchards of the Citrus Experiment Station at a period when the plantings were very limited in extent, it is probable that it came from but two or three trees at most and it is possible and even probable that it all came from one single tree. There does not seem to have been sufficient genetic variation in the population with variants removed to insure any permanent influence on the scions as indicated by size characters, and it seems reasonable to conclude, therefore, that this population is probably to be considered as nearly homogeneous genetically.

The seedlings of this population when measured just before the buds were inserted, exhibited a range of variation in area of cross section of trunk of 25.43 per cent. Apparently this variation is mainly environmental.

Soil variability in the experimental orchard (orchard 1-Z) might be expected to influence the variability in both size and yield, and the plotting of the orchard by tree yield shows that certain areas are evidently better than others, but apparently these differences in soil fertility cannot be interpreted as responsible for the final results obtained.

The seedlings used as stocks in this experiment were in general somewhat larger when budded than is usual in ordinary nursery practice, and it was suggested that the largest ones were probably too large to heal favorably and react equally well on the buds. A large seedling, when budded, does not give a stimulus to growth proportional to its size, as might be expected, since the growth of the bud for a considerable period draws mainly on one side only of the stock. A bud inserted in a stock 1 inch in diameter may not fully grow over the cut trunk of the stock for two years or more, and meanwhile the scion is drawing its supply of soil solutes mainly from a limited part only of the seedling root system, and is very imperfectly supplying the carbohydrate requirements of the large root system.

It is also important to remember that the shock caused by cutting off the top in forcing the buds is likely to be comparatively more severe with the large seedlings than with the smaller ones, and a much longer time may be required to reestablish the normal balanced relation between root and budling shoot than would occur when a smaller seedling is budded. Under such conditions it might be assumed that the large seedling, even if inherently better than a smaller one, would not fully show the influence of its size during a period of possibly several years after budding. The inquiry thus arose as to whether the lack of correlation between seedling size and size of 8-year orchard trees might not be due partially to this influence. If this were true it would seem that the effect might be detected by dividing the seedling population according to size into quartiles, and studying the relations: the small and medium-sized seedlings might be expected to give better results than the very large ones.

Table 5 gives the constants after the division of the population, exclusive of variants, into quartiles based on the trunk area of the seedlings in 1921.

TABLE 5  
RELATION OF INDIVIDUALS WITHIN QUARTILES BASED ON SEEDLING SIZE, WITH  
VARIANTS EXCLUDED

Constants	First quartile	Second quartile	Third quartile	Fourth quartile
Population in each quartile.....	86	87	87	86
Mean trunk area of seedlings, 1921, in sq. cm.....	2.47±0.030	3.31±0.008	4.19±0.030	5.54±0.065
Mean trunk area of orchard trees, 1929, in sq. cm.....	78.83±0.969	80.83±0.654	83.82±0.866	80.09±0.821
Coefficient of variability, orchard trees, 1929, in per cent.....	16.92±0.894	11.19±0.579	14.29±0.745	13.99±0.733
Coefficient of correlation, area 1921 seedlings with 1929 trees.....	-0.058±0.073	+0.0012±0.075	-0.230±0.070	-0.251±0.069

The examination of the mean size of the orchard trees in each quartile shows that the small and large trees in 1929 are very nearly equally distributed in the different quartiles and that the percentage of variability also is practically the same in each. The correlations in the first and second quartiles are in both instances so small as to indicate no correlation, but those in the third and fourth quartiles,  $-0.230 \pm 0.070$  and  $-0.251 \pm 0.069$ , are possibly sufficiently large in relation to their probable errors to indicate a tendency for the large seedlings in the third and fourth quartiles to produce somewhat smaller 8-year-old orchard trees. Therefore this may indicate a slight holdover influence of a detrimental effect from budding too large seedlings. The writer is not inclined to consider these figures as more than a suggestion in connection with future work.

It must be granted that the evidence presented here indicates that there is no apparent consistent relation between the size of the stock seedlings after the variants are eliminated and the size of the orchard trees in later years after they have reached an age of 8 years. If this is recognized as the true interpretation of the results, and it seems a fair conclusion from the evidence presented, then any nursery selection based upon size of stock seedlings existing after the so-called variants are excluded, would seem to be valueless.

It should be remembered, however, that the total yield of the trees during the first five seasons after they came into bearing correlated with the trunk area of the stock seedlings gave a small positive correlation of  $+0.135 \pm 0.035$ . It may be that even this low correlation indicates a sufficient influence of seedling size to justify the discarding of the small seedlings before budding. A study of the annual tree yields of the crops for the period from 1925-26 to 1929-30 and a correlation of each with stock seedling size in 1921 as shown by area of trunk cross section gave the following data:

Crop season	Mean annual yield per tree, in pounds	Coefficient of variation, in per cent	Coefficient of correlation with 1921 seedlings
1925-26.....	18 51 $\pm$ 0.278	41 37 $\pm$ 1.235	+0.291 $\pm$ 0.033
1926-27.....	53.32 $\pm$ 0.670	34.62 $\pm$ 0.987	+0.160 $\pm$ 0.035
1927-28.....	42.68 $\pm$ 0.890	57.42 $\pm$ 1.894	+0.147 $\pm$ 0.036
1928-29.....	101.35 $\pm$ 1.527	41.50 $\pm$ 1.232	+0.157 $\pm$ 0.036
1929-30....	62.22 $\pm$ 0.930	41.59 $\pm$ 1.235	+0.072 $\pm$ 0.037

A comparison of the coefficient of variation in yield for the different seasons shows no indication that the variation in tree yield is decreasing as the trees grow older. This is in marked contrast to the

gradual decrease in the percentage of variation that took place in all tree size characters measured.

There was a fairly significant correlation in 1925-26, which became less in the succeeding years and in the last year of the five-year period was entirely insignificant.

The same population treated in another way by a direct selection based on the stock diameter of the seedlings indicates that the gain in yield during the period concerned was sufficient to justify a fairly severe selection. (See discussion on page 63.)

#### RELATION OF SIZE OF BUDLINGS TO SIZE OF 8-YEAR-OLD ORCHARD TREES, AS SHOWN BY TRUNK AREA

In table 6 the various statistical constants are given for the size of budling as shown by area of trunk cross section when 1 year old in the nursery, at the time of digging in the spring of 1922, as compared with the size of scion trunk of the orchard trees November 1, 1929, at the close of the eighth season of growth in the orchard.

In table 6 the data is divided on the basis of the grading of the seedlings *at the seed bed* into firsts and seconds according to size, and later *at time of budding* into normal and variant types. These segregations must be kept clearly in mind to understand the discussion. An examination of columns 1, 2, and 3 of this table shows that when the entire population is compared with the firsts only, and with seconds only, the mean size of the firsts is greater than that of the seconds, while also, the standard deviation is less for the firsts, than for the seconds. This seems natural since the seconds contain the larger proportion of the dwarfed variant types. The coefficient of variability of the three groups follows the same rank as the standard deviation. It is interesting to note that in each of the three groups the 1-year-old budlings show a much greater coefficient of variation than the 8-year-old trees.

The coefficients of correlation in which greatest interest centers are  $+0.622 \pm 0.021$  for the entire population,  $+0.411 \pm 0.037$  for the firsts, and  $+0.743 \pm 0.025$  for the seconds. These correlations are sufficiently high in each case to be considered markedly significant. It is very evident that either with the entire population or with merely the firsts or the seconds when the variants are included, there is a strong probability that a large nursery tree will tend to produce a relatively large orchard tree and that a small nursery tree will tend to produce a small orchard tree.

TABLE 6  
COMPARISON OF SIZE OF BUDLINGS WITH SIZE OF ORCHARD TREES GROWN FROM THEM  
(Area of cross section of scion trunk at 1 year, 1922, with area at 8 years, 1929)

Constants	Age	Variants included				Variants excluded			Variants only from both firsts and seconds
		Entire population	Firsts only (large)	Seconds only (small)	Entire population	Firsts only (large)	Seconds only (small)		
		<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	
Population.....		389	241	148	346	231	115	43	
Mean trunk area in sq. cm	1 year	2 45±0 032	2 56±0 032	2 27±0 065	2 69±0 025	2 65±0 027	2 77±0 050	0 53±0 037	
	8 years	75 76±0 688	78 12±0 685	71 92±1 395	81 05±0 422	79 91±0 535	83 38±0 647	33 20±2 278	
Standard deviation, in sq. cm.....	1 year	0 94±0 023	0 74±0 024	1 17±0 046	0 68±0 017	0 62±0 019	0 79±0 035	0 36±0 026	
	8 years	20 11±0 487	15 78±0 500	25 18±0 987	11 63±0 298	12 07±0 379	10 29±0 458	22 13±1 609	
Coefficient of variability, in per cent	1 year	38 42±1 058	29 05±0 996	51 63±2 503	25 43±0 692	23 32±0 771	28 55±1 370	67 29±6 756	
	8 years	26 54±0 086	20 20±0 666	35 02±1 532	14 35±0 375	15 10±0 485	12 34±0 557	66 67±6 665	
Coefficient of correlation, budling area with tree area		+0 622±0 021	+0 411±0 037	+0 743±0 025	+0 182±0 034	+0 142±0 042	+0 232±0 058	+0 390±0 087	

In view of the fact that the early study of the stock seedlings used revealed the presence of numerous variants, or types differing from the normal standard type of the population, it is important to carry the analysis further and determine what effect these variant types have had on the results. The second part of table 6 gives the same statistical constants for the same groupings of the population as those given in columns 1, 2, and 3, but with the trees known to be on variant stock seedlings excluded from consideration.

By comparing columns 4, 5, and 6 of table 6 it may be noted that when the variants are excluded the area of cross section of budling trunk of the seconds (i.e., the group grown on seedlings graded at the seed bed as seconds in size), at 1 year of age in the nursery gives the largest mean size  $2.77 \pm 0.050$  sq. cm, while the mean for the firsts is slightly less, being  $2.65 \pm 0.027$  sq. cm, and that for the entire population exclusive of variants is  $2.69 \pm 0.025$  sq. cm, an intermediate figure. It is also interesting to note that the same relation of size exists at the end of the eighth growing season in the orchard when the means are for seconds,  $83.38 \pm 0.647$  sq. cm; entire population,  $81.05 \pm 0.422$  sq. cm; and firsts,  $79.91 \pm 0.535$  sq. cm.

The standard deviation for the trunk areas of the 1-year-old budlings is greater for the seconds than for the firsts, while after 8 seasons in the orchard the same population shows the standard deviation greater for the firsts than for the seconds.

The coefficient of variability for the trunk areas of the 1-year-old budlings is greater for the seconds than for the firsts but after 8 seasons this order is reversed.

The tendency of the seconds, as shown by the mean area of budling trunk when the variants are excluded, is to be rather larger than the firsts, and they also seem to show a slightly greater uniformity as indicated by a lower standard deviation and a smaller coefficient of variability.

It will be seen later that when the variants are excluded, the seconds are also slightly superior to the firsts as shown by a somewhat larger mean volume of top and total 5-year yield and by a lower standard deviation and coefficient of variability for each of these characters.

Even though this result is obtained with the exclusion of the variants, which were most numerous among the seconds, it is the reverse of what would generally have been expected.

As this result was obtained with two segregated portions of the same population, after the variants were excluded, the seedling root-

stocks were doubtless almost wholly of apogamic origin, and thus approximately of the same genetic constitution. Apparently, therefore, it may be concluded that this slight superiority of the seconds is due to the favorable influence of the greater space available for their development owing to the high mortality among the variants which were most numerous in this group (see fig. 2, orchard 1-Z), or possibly to the slight detrimental effect caused by the first-grade seedlings, being somewhat too large when they were budded (see table 5). It does not seem probable that the difference is to be considered as significant.

The coefficients of correlation in these three groupings of the population with the variants excluded (columns 4, 5, and 6 of table 6), are for the entire population  $+0.182 \pm 0.034$ ; for the firsts,  $+0.142 \pm 0.042$ ; and for the seconds,  $+0.232 \pm 0.058$ . While these correlations are small and barely significant, they all show the general tendency of the large scions in the nursery to produce the large orchard trees and add to the evidence favoring this conclusion.

Statistical constants for the variants alone are given in table 6, column 7. The trees all average much smaller than those in the other groupings of the population, the mean size of 1-year budlings being only  $0.53 \pm 0.037$  sq. cm in comparison to  $2.69 \pm 0.025$  sq. cm for the entire population exclusive of variants. The 8-year-old variants had a mean size of  $33.20 \pm 2.278$  sq. cm, while the population exclusive of variants had a mean size of  $81.05 \pm 0.422$  sq. cm. This illustrates very clearly the dwarfing effect that the variant seedlings produce in the scions grown on them and shows why the variants should be eliminated and not used as stocks. The variants also, as would probably be expected, exhibit a high standard deviation and coefficient of variation.

It will be noticed that the population of variants gave a correlation of  $+0.390 \pm 0.087$  between nursery size and orchard size of tree. This perhaps has no practical bearing on the problem of nursery selection, as all the variants should certainly be discarded, but it does show that within this limited special population the size of the budling tends to influence the size of the budded tree, and evidence of such an influence, if it exists generally, is of interest.

In view of the general tendency of the percentage of variation and the degree of correlation with seedling size to decrease as the trees grow older, as shown by the comparison of size of stock seedlings in 1921 with the size of orchard trees in 1929 (table 4), and of budlings in 1922 with orchard trees in 1929 (table 6), it is desirable



to know what occurs when size of budling is considered in relation to size of orchard trees at various periods. When the total population exclusive of variants is taken (346 trees), and the area of budling trunk when 1 year old at time of transplanting in 1922 is correlated with area of scion trunk of the orchard trees in 1924 when 2 years old, in 1927 when 6 years old, and in 1929 when 8 years old the coefficients of correlation are, for 1924,  $+0.358 \pm 0.032$ ; for 1927,  $+0.170 \pm 0.036$ ; and for 1929,  $+0.182 \pm 0.034$ .

Evidently there is a decreasing correlation until the sixth year, when the trees apparently reach a condition approaching the normal variability of mature trees as the correlation in the eighth year is practically equal to that of the sixth year.

#### RELATION OF SIZE OF BUDLINGS TO SIZE OF 8-YEAR-OLD ORCHARD TREES, AS SHOWN BY TOP VOLUME

In the preceding section the correlations are given between the size of the budlings as indicated by area of cross section of scion trunk at time of transplanting, with the area of trunk of the orchard trees when 8 years of age and at intermediate periods. As any single measurement of size is subject to considerable variation and may not be a true index of the existing condition, it is desirable where possible to use other measurements of size as a check on the results. The volume in cubic feet of the tops of the trees in the same population was obtained in June, 1930, by the use of a standard fumigation tent, in order to use this index of size for comparison with trunk area and other data.

In table 7 statistical constants are given, similar to those of table 6 but comparing the size of the scion trunk of the 1-year budlings at the time of digging with the volumes of the tops of the same trees 8 years later in the orchard.

It will be seen from an examination of columns 1, 2, and 3 of this table that the mean size of top is greatest for the first-grade trees, least for the second grade, and intermediate for the entire population. The standard deviation and coefficient of variability are both less for the firsts than for the seconds. It cannot be stated whether or not the coefficient of variability decreases for volume of top as the trees grow older, as does the variability in area of scion trunk, because measurements of the volume of top were not taken for the young trees.

The coefficient of correlation between the trunk area of the young trees and the top volume of the 8-year-old orchard trees of  $+0.598$

TABLE 7  
COMPARISON OF TRUNK AREA OF BUDLINGS IN 1922 WITH TOP VOLUME OF 8-YEAR-OLD ORCHARD TREES IN JUNE, 1930

Constants*	Top volume of 8-year-old orchard trees, in cu. ft.						
	Variants included			Variants excluded			Variants only from both firsts and seconds
	Entire population 1	Firsts only (large) 2	Seconds only (small) 3	Entire population 4	Firsts only (large) 5	Seconds only (small) 6	
Population.....	389	241	148	346	221	115	43
Mean, in cu. ft.....	503.70±5.971	521.80±6.178	472.80±10.534	546.90±4.182	533.75±5.400	568.00±6.224	158.20±19.260
Standard deviation, in cu. ft.....	174.60±4.225	142.35±4.512	190.15±5.745	115.20±2.949	121.90±3.823	93.95±4.403	187.17±13.608
Coefficient of variability, in per cent.....	34.66±0.334	27.28±0.927	40.22±1.814	21.08±0.563	22.75±0.731	17.42±0.798	118.31±16.766
Coefficient of correlation with budling area in 1922.....	+0.598±0.022	+0.386±0.040	+0.836±0.017	+0.202±0.065	+0.154±0.044	+0.288±0.037	+0.264±0.088

\* The constants for the same groupings of the population of 1922 budlings are given in table 6.

TABLE 8  
COMPARISON OF SCION TRUNK AREA OF BUDLINGS IN 1922 WITH YIELD OF ORCHARD TREES

Constants*	Total yield of 5-year period (1925-28 to 1929-30)						
	Variants included			Variants excluded			Seconds only (small) 6
	Entire population 1	Firsts only (large) 2	Seconds only (small) 3	Entire population 4	Firsts only (large) 5	Seconds only (small) 6	
Population.....	389	241	148	346	231	115	
Mean yield, in lbs. per tree.....	256.96±3.744	257.80±4.422	255.32±6.600	277.18±3.432	265.78±4.207	300.10±5.121	
Standard deviation, in lbs.....	109.48±2.649	101.88±3.230	119.14±4.670	94.34±2.420	94.90±2.982	89.36±3.677	
Coefficient of variability, in per cent.....	42.62±1.204	39.52±1.435	46.66±2.191	34.11±0.989	35.73±1.257	29.78±1.438	
Coefficient of correlation with budling trunk area in 1922.....	+0.517±0.025	+0.335±0.040	+0.703±0.029	+0.233±0.034	+0.154±0.014	+0.244±0.060	

\* The constants for the same groupings of the population of 1922 budlings are given in table 6.

$\pm 0.022$  for the entire population,  $+ 0.386 \pm 0.040$  for the firsts, and  $+ 0.836 \pm 0.017$  for the seconds clearly indicates a strong tendency of the large nursery trees to produce a fairly high percentage of the large orchard trees and vice versa, as judged by top volume.

It is important also to consider the population with the variants excluded, as was done in the preceding section. When the entire population exclusive of variants is taken, and nursery size of budling trunk compared with volume of top at 8 years (columns 4, 5, and 6, table 7) the combined population gives a coefficient of correlation of  $+ 0.202 \pm 0.035$ , while the firsts give a correlation coefficient of  $+ 0.154 \pm 0.044$ , and the seconds  $+ 0.288 \pm 0.057$ . It is thus shown again that the variants in the population (which are comparatively small in size in the early stages of growth, and in general have the effect of severely dwarfing the scions grown on them) are largely responsible for the high positive correlations shown in every case where any grouping of the population is taken with the variants included.

It will also be noticed in comparing populations of columns 4, 5, and 6 in table 7 (with variants excluded) that the seconds show, for top volume, a larger mean size and a smaller standard deviation and coefficient of variation than the firsts, a similar condition to that shown by the same populations when area of trunk of the orchard tree was used as the indicator of size. The differences, however, are comparatively small and probably have no significance.

It becomes increasingly evident from the data in this table that the segregation of the seedlings at the seed bed into firsts (large) and seconds (small) had no effect other than to segregate the largest proportion of the variants with the seconds.

The most important constant in this table is the correlation of  $+ 0.202 \pm 0.035$  between the size of the budling trunks of the population without variants and the top volume of 8-year-old orchard trees. This indicates a persisting influence of budling size on orchard-tree size even after the variants are eliminated.

In order to show the relation existing between trunk size and volume of top measurements taken at about the same age, the areas of scion trunk taken in November, 1929, were compared with the measurement of top volumes, taken in June, 1930. Here when the entire population of 389 trees is considered the correlation is  $+ 0.923 \pm 0.006$ , and when the variants are excluded the correlation is  $+ 0.817 \pm 0.013$ . These high correlations indicate a very close relation between the size of the trunk and the size of the top at about the same period of development.

## RELATION OF SIZE OF BUDLINGS TO YIELD OF ORCHARD TREES

While in this experiment there has been a general tendency for the large budlings to produce large orchard trees, it is of even more practical interest to know whether there is any direct correlation between the size of the budlings and the yield of the trees in the orchard. Interest centers in the production of fruit, and it matters little what relation one character bears to another unless in some way this relation bears on the quantity, quality, or grade of the fruit produced. The trees in this experiment have been producing some fruit since the year 1924-25 and the individual tree production has been recorded for a period of 5 years (1925-26 to 1929-30 inclusive). The yield of the first 5 years in the life of the orchard tree can scarcely be taken as indicating its final relative position as to yield, yet it will be granted that even the first 5 years of the fruiting period is important and is probably an indication of later performance. Table 8 gives the coefficients of correlation between area of cross section of scion trunk of the nursery trees and the total 5-year yield of the same trees, with the populations grouped as in the preceding tables.

It is of particular interest to note in table 8 that with the 389 trees considered in this experiment all segregations of the population studied give some degree of positive correlation between the original size of the nursery tree and the total yield produced. When the entire population is considered this correlation is found to be  $+0.517 \pm 0.025$ ; for the firsts only,  $+0.335 \pm 0.040$ ; and for the seconds only,  $+0.703 \pm 0.029$ . These correlations are sufficiently large to show conclusively that there is a very marked relation between the size of the nursery tree and its probable yield as an orchard tree when the variants are included.

When the variants are excluded from the population, these correlations become, for the entire population  $+0.233 \pm 0.034$ ; for the firsts,  $+0.154 \pm 0.044$ ; and for the seconds,  $+0.244 \pm 0.060$ . These are smaller and less significant correlations, but they do indicate a tendency for the large nursery trees (budlings) to produce high-yielding orchard trees and vice versa, even after the variants have been excluded. They also emphasize the effect of the variants on yield.

It will also be noticed that populations 4, 5, and 6, when the variants are excluded, give reversals in mean, standard deviation, and coefficient of variation between the different groups of firsts and seconds similar to those shown in tables 6 and 7.

# CHANGES IN INTERRELATIONS OF SIZE AND YIELD AS TREES INCREASE IN AGE

Attention has been directed in preceding sections to the decrease that gradually takes place in the range of variation and also in the coefficients of correlation of the trunk areas (cross section of trunk) of the seedlings in 1921 and of the budlings in 1922 compared with the orchard trees at different periods as the trees grow older. In order to obtain a clear picture of what is taking place, it is interesting to compare these figures with interperiod and interannual correlations. It is important to know whether the trees that push ahead rapidly and attain large comparative size, continue to maintain this larger size and possibly give larger yields as a result.

The data giving the relation of the early trunk area of the seedlings and budlings of the population exclusive of variants to the later trunk areas of the orchard trees is summarized for easy comparison in table 9.

TABLE 9  
RELATION OF TRUNK AREA OF SEEDLINGS AND BUDLINGS TO TRUNK AREA OF  
TREES IN LATER YEARS  
(Population, exclusive of variants, 346 trees)

Year	Age, years or seasons of growth	Coefficient of variation, in per cent	Correlation with 1922 budling size
1921 seedlings.....	3	33.05±0.934	+0.549±0.026
1922 budlings.....	1	25.43±0.662	
1924 trees (April).....	2	18.43±0.488	+0.358±0.032
1927 trees (September).....	6	15.92±0.417	+0.170±0.035
1929 trees (November).....	8	14.35±0.375	+0.182±0.034

An examination of these data shows the rather rapid smoothing out of the variation and the decrease of the correlation between original and final size. This might lead to the conclusion that there is no permanent relation of size and that there might possibly be a fluctuation in different years. Interperiod correlations, however, show that there is apparently an increasingly stronger correlation from one period to the next as the trees grow older. This is indicated by the following data, consisting of correlations between size as indicated by area of trunk section for different periods, for the population exclusive of variants (346 trees):

Correlation coefficient	
1922 budlings with 1924 trees	+ 0.358 ± 0.032
1924 trees with 1927 trees	+ 0.618 ± 0.023
1927 trees with 1929 trees	+ 0.781 ± 0.014

It will be seen that the correlation coefficients increase very materially as time goes on. These figures tend to confirm the results of Sax and Gowen (1923), Collison and Harlan (1930), and others, as to the permanency of size relations in orchard trees.

It has been found that the original trunk area of the budlings, exclusive of variants, correlated with the 1929 scion trunk area of orchard trees gave only the low correlation of  $+0.182 \pm 0.034$ , and that the seedling trunk areas at the time of budding when compared with 1929 seedling trunk areas gave a negative correlation of  $-0.054 \pm 0.032$ , while during the same series of years the interperiod correlations reached the relatively high positive coefficient of  $+0.781 \pm 0.014$ . From this it would seem that the high degree of correlation exhibited in the later periods of growth is most likely due to some other influence than variations in the rootstocks. It would appear probable that it is caused by some more or less permanent and continuously acting environmental difference.

In the studies of Parker and Batchelor (1932) it was found that tree size as shown by trunk cross section compared with tree yield in the same year gave gradually increasing correlations during the period when the trees were from 6 to 10 years of age. These correlations, for the respective years, were  $+0.109$ ,  $+0.233$ ,  $+0.247$ ,  $+0.278$ , and  $+0.322$ . The population of 346 trees with variant types eliminated, which is under consideration in the present discussion, gave a correlation of  $+0.257 \pm 0.034$  between 1927 trunk area and with 1927 tree yields; and a similar comparison in 1929 gave a correlation of  $+0.261 \pm 0.033$ .

The experiment has not been under way long enough to afford opportunity to obtain interannual yield correlations that can be considered of very great value in the analysis of conditions. Yields have been recorded for 5 years, but those for the first two years of this period, when the trees were 4 and 5 years old respectively, were too small and variable to be given much consideration. For the next 3 fruit years, when the trees were approximately 6, 7, and 8 years old, the mean yields, coefficient of variability, and correlation coefficients, were as follows:

Year	Mean yield per tree	Coefficient of variability	Correlation with yield of preceding year
	<i>pounds</i>	<i>per cent</i>	
1927-28	42.68 $\pm$ 0.890	57.42 $\pm$ 1.893	.....
1928-29	101.35 $\pm$ 1.527	41.50 $\pm$ 1.232	+0.724 $\pm$ 0.018
1929-30	62.22 $\pm$ 0.939	41.59 $\pm$ 1.235	+0.532 $\pm$ 0.026

The decrease in the coefficient of variability in the last two years (1928-29 and 1929-30) might be taken as indicating that the differences in yield between the various trees have gradually smoothed out as the trees grew older. The variability in preceding years, however, as given on page 39, does not support this assumption.

#### SUGGESTIVE RESULTS FROM THIS EXPERIMENT

One of the outstanding features of this experiment is the uniformity of the results obtained in comparing budling and orchard-tree size regardless of the measure used in the calculations. Within this lot of 389 trees, the coefficients of correlation obtained between area of cross section of trunk of the budlings and area of cross section of trunk, volume of top, and total yield of the 8-year-old orchard trees, were of about the same value for each segregation of the population; they differed little more than would be expected from the unavoidable errors of measurement. The conformity of these results appears to the writer to be so striking and important that the coefficients of correlation for the various measurements and segregations of the population are brought together in table 10 (cols. 3, 4, and 5) where they can be more easily compared. In column 2 of this table the same data are also given for nursery seedlings correlated with 1-year budling size, though these are naturally very different, and are to be considered only as showing the relation in size between the seedlings and budlings of the different groups.

TABLE 10

COEFFICIENTS OF CORRELATION BETWEEN SIZE OF NURSERY TREES AND SIZE OF ORCHARD TREES GROWN FROM THEM AS SHOWN BY AREA OF SCION TRUNK, VOLUME OF TOP, AND TOTAL 5 YEAR YIELD

Population	Trunk area of seedlings, 1921, with 1-year budlings, 1922	Trunk area of 1-year budlings with trunk area of 8-year trees	Trunk area of 1-year budlings with top volume of 8-year trees	Trunk area of 1-year budlings with total 5-year yields
1	2	3	4	5
A Entire population	+0 736±0 016	+0 622±0 021	+0 598±0 022	+0 517±0 025
B First grade only	+0 663±0 025*	+0 411±0 037	+0 386±0 041	+0 335±0 040
C Second grade only	+0 823±0 018*	+0 743±0 025	+0 836±0 017	+0 703±0 029
D Entire population exclusive of variants	+0 549±0 026	+0 182±0 034	+0 202±0 035	+0 233±0 034
E First grade only exclusive of variants	+0 589±0 029*	+0 142±0 042	+0 154±0 044	+0 154±0 044
F Second grade only exclusive of variants	+0 545±0 045*	+0 232±0 058	+0 288±0 057	+0 244±0 060

\* These correlations are not given elsewhere

A comparison of the items in each of the lines of this table will indicate very clearly the great uniformity exhibited in each segregation of the population for each character measured. It seems that this must be interpreted as strong confirmation that, with this population, under the conditions of the experiment, the measurements recorded and the constants derived from them must be considered as fairly accurate.

It seems logical to conclude that, if an entire population including variants is considered where uniform buds of a scion variety have been used as under the conditions of this experiment, the chances are strong that a large budling or nursery tree will tend to produce a relatively large, high-yielding orchard tree, and that a small budling is most likely to produce a relatively small and low-yielding orchard tree.

It seems clear that this effect is largely, but not entirely, due to the fact that the great majority of the small low-yielding orchard trees are such because they have been grown on variant, off-type stocks, and that at least the great majority of such variants in the seedling stocks in the population studied, could be recognized in the nursery by a careful observer, and eliminated.

It is of primary importance to note that the great majority of these variant types were found among the second-grade or small seedlings and would have been largely eliminated by a selection based on size when they were dug from the seed bed. It should also be noted that a selection of budlings based on size would have eliminated most of the variants. That the elimination of these variant types is of first importance cannot be doubted.

The analysis of the data also shows that in the population remaining after the variants have been excluded, there is still a small degree of correlation between budling size and the size and yield of orchard trees at 8 years of age. This correlation in the reduced population is still large enough to indicate a tendency for the large selected budling trees to give the better results.

In view of the fact that the second-grade trees, when the variants were excluded, gave a slightly larger mean size after 2 years in the nursery and also after 8 years in the orchard than did the first-grade trees, it may be concluded that the segregation into first and second-grade seedlings at the seed bed, based on size, did not accomplish anything other than to place the variants mainly in the second grade. It was these dwarfed, variant types that accounted also for the strong correlation between budling size and orchard size, when the entire



population was considered. It would appear that the seedlings in the seed bed were grown under such crowded conditions that their size is not a true indication of their inherent vigor, other than that the variants are mainly weak and thus small. Sax (1928) found the same to be true with apple seedlings, and states that "The size of the seedling as it comes from the wholesale nursery has little or no relation to the size of the 1-year-old nursery tree. Large seedlings did not produce larger whips than small seedlings. Evidently the size of a 1-year-old seedling as commonly grown, is so much influenced by crowding and other environmental factors that size is no indication of its hereditary vigor." After the seedling apple stocks have grown under nursery conditions with equal spacing for a year and reach the age for budding, their size was found by Sax (1924) to be a significant indication of their future growth, and he states "Correlations were obtained between size of the French crab seedlings and the size of the nursery trees grown on these seedling roots. The correlations between size of seedling and size of the one-year whips was found to be 0.36, 0.38, 0.26, and 0.43, respectively, for the four varieties, McIntosh, Ben Davis, Delicious, and Northern Spy. The correlation between seedling size in the fall of 1922 and the size of the 2-year nursery trees in 1924, was found to be 0.42, 0.39, 0.38, and 0.45 for the above varieties. In all cases the size of seedling root seemed to have slightly more influence on the nursery trees as they became older."

Sax's results indicating that "the size of the seedling root seemed to have slightly more influence on the nursery trees as they became older" do not seem to hold in citrus. In the citrus experiment under consideration, the correlation between budding size and size of scion trunk of orchard trees gradually becomes smaller as the trees grow older, at least up to the close of the 1929 season when the trees were 8 years old, and the coefficients of variation gradually become less in the population as a whole. Sax's results were apparently obtained with young trees growing continuously in the same place and it seems probable that the increased correlations observed are to be interpreted as mainly due to soil variations just as with the increased interperiod correlations obtained by the writer.

It should also be remembered in comparing these results that apple seedlings because of cross-pollination are likely to be highly variable, but that citrus seedlings because of apogamy are likely to be much less variable.

In the case of the reactions of the French crab seedlings with which Sax's results were obtained, probably a considerable number of the variant seedlings that would give extreme dwarfing had been eliminated at the wholesale nursery; and yet the results show that, as judged by size at the end of a year in the nursery, the seedlings gave a fairly significant coefficient of correlation.

With cherries, Burkholder and Green (1929) found that with a population of 1,191 Mahaleb cherries budded with Montmorency, seedling size at time of budding correlated with size of budlings at end of 1 year's growth gave a correlation coefficient of  $+0.685 \pm 0.0158$ . The same correlation for the citrus population of 387 trees under consideration was  $+0.736 \pm 0.016$  (see table 3).

In the study of two apple orchards of different varieties over a period of 20 years, Collison and Harlan (1930) found that the trees maintain their comparative size relations to a marked degree, but they also found that "variability in both yield and diameter growth becomes less with increasing age of tree." These results were obtained with orchard trees of considerable age and size when the records began, while in the present experiments the study started with the planting of the seed, and has continued only to the eighth year in the orchard. It is significant, however, that with both apples and citrus the variation shown during the first part of the period persists. As is pointed out elsewhere, buds are variable in time of starting, and thus in the early stages of budling growth the range in comparative size is very great; but it gradually becomes less evident as the budling tops grow older. One of the factors contributing to the increased variability of young trees is believed to be the distinct shock which the rootstock suffers at the time of budding and topping, which results in a great lack of balance between the root and top. The normal balance is more slowly regained if the rootstock is large.

Relative to the coefficient of correlation between budling size in 1922 and the size of the orchard trees at different periods of growth, it will be seen that this decreases from  $+0.358 \pm 0.032$  in 1924 to  $+0.170 \pm 0.035$  in 1927, and in 1929 is  $+0.182 \pm 0.034$ , an insignificant increase over that for 1927. The figures would suggest that the coefficient of correlation may decrease for a certain period until an equilibrium is established between the various factors affecting growth.

## EFFECT OF BUDLING SIZE ON THE SIZE AND YIELD OF ORCHARD TREES IN A SELECTED POPULATION ON SWEET-ORANGE STOCKS<sup>13</sup>

Washington Navel orange trees on sweet-orange stocks grown in the fertilizer experiments of the Citrus Experiment Station furnish interesting data on the continued maintenance of comparative size. This orchard contains 1,506 normal Washington Navel orange trees that were planted in the spring of 1917. (See page 15 for statement of early history of these trees.)

These trees were grown on sweet-orange stocks taken from an especially uniform, good seed bed from which at the time of digging some 10 per cent of the total number of seedlings were discarded as being too small to plant. The seedlings chosen were grown in a nursery at the Station, given uniform treatment, and budded with carefully chosen buds from good trees of known performance record. At the time of budding all noticeably small and off-type seedlings were also eliminated. When the budlings were transplanted into the permanent experimental orchard, the large ones only were chosen for the planting. No exact record was made of the number of small and medium-sized trees that were discarded, but the writer assisted in choosing the trees for the planting and estimated that about 30 per cent of the total number of budlings were discarded as being too small to meet the requirements of size. This selection probably eliminated all or nearly all of the trees that were propagated on variant seedlings. Extra care was taken to treat these trees as uniformly as possible during the period of 10 years after planting, before the differential fertilizer treatments were applied.

The trunk measurements recorded were made first in 1918, approximately 1 year after the trees were planted in the orchard. They have been measured at regular intervals since that time, and the yield has been recorded annually.

Table 11 gives certain statistical constants obtained from a study of the data of this orchard. The data for area of cross section of scion trunk in 1918 is compared with that of the area of scion trunk

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<sup>13</sup> The measurements of size and yield used in the correlations reported here were taken from the records of the fertilizer experiments of the Citrus Experiment Station, and are used with the kind permission of Drs. L. D. Batchelor and E. R. Parker.

in 1926, and also with that of the average total yield of each tree during a period of 7 years, from the crop of 1920-21 to that of 1926-27 inclusive.

TABLE 11

COMPARISON OF SIZE OF 1 YEAR-OLD WASHINGTON NAVEL ORANGE TREES ON SWEET ORANGE STOCKS WITH SIZE AND YIELD OF THE SAME TREES AFTER 9 GROWING SEASONS

(A selected population of 1,506 from which variant seedling stocks had been removed)

Statistical constants	Area of scion trunk at 1 year, 1918	Area of scion trunk at 9 years, 1926	Average total yield per tree during 7 years. Crops of 1920 21 to 1926 27
Mean	6 23±0 021 sq cm	127 18±0 311 sq cm	776 38±2 318 lbs
Standard deviation	1 20±0 015 sq cm	17 89±0 220 sq cm	133 19±1 638 lbs
Coefficient of variability	19 13±0 244 per cent	14 07±0 176 per cent	17 15±0 217 per cent
Coefficient of correlation with 1-year-old trees		+0 158±0 017	+0 229±0 016

An examination of the data in table 11 shows that the size of the trees became slightly less variable as they grew older, the coefficient of variability for the 1-year-old trees being  $19.13 \pm 0.244$  per cent in comparison with  $14.07 \pm 0.176$  for the 9-year-old trees. The correlation coefficient of  $+0.158 \pm 0.017$  between the size at 1 and 9 years of age, is small but significant and indicates a general tendency for the trees to retain the relative position of size held at the time of the measurement when the trees were only 1 year old.

When the average yield per tree during the 7-year period is compared with the size of the 1-year-old trees, as shown by area of trunk section, a correlation coefficient of  $+0.229 \pm 0.016$  is obtained.

In this population of Washington Navels on stocks of sweet-orange seedlings, the degree of selection practiced would correspond very closely to that of the population of the preceding experiment with variants excluded, the constants for which are given in column 4 of tables 6, 7, and 8. It is interesting to note how very close together the corresponding constants are, indicating that the possible scionic influence due to size of stock seedlings selected is likely to be about the same with sweet as with sour stocks. To show more clearly the similarity in magnitude of the corresponding constants for the two populations, table 12, in which the two populations are distinguished by their stocks, will be of assistance.

Chief interest in the data assembled in table 12 centers in the fact that for the two populations on sour stock and sweet stock the

coefficients of correlation between size of budling and size of orchard tree ( $+0.182 \pm 0.034$  and  $+0.158 \pm 0.017$  respectively), and between the size of budling and total yield ( $+0.233 \pm 0.034$  and  $+0.229 \pm 0.016$  respectively) are so nearly alike. In view of this fact it would seem probable that these degrees of correlation may be taken as approximately the normal correlation to be expected with similarly selected citrus trees under such conditions on either sweet or sour-orange rootstocks.

TABLE 12

COMPARISON OF STATISTICAL CONSTANTS FOR TWO POPULATIONS OF WASHINGTON NAVAL ORANGES WITH APPROXIMATELY EQUAL DEGREE OF SELECTION (VARIANTS EXCLUDED), BUT ONE ON SOUR STOCK AND THE OTHER ON SWEET STOCK\*

Stock	Coefficients of variability			Coefficients of correlation	
	Trunk area of budlings	Trunk area of orchard trees	Yield of orchard trees	Trunk area of budling with trunk area of orchard trees	Trunk area of budling with total yield of orchard trees
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
Sour	25.43 $\pm$ 0.692	14.35 $\pm$ 0.375	34.11 $\pm$ 0.969	+0.182 $\pm$ 0.034	+0.233 $\pm$ 0.034
Sweet	19.13 $\pm$ 0.244	14.07 $\pm$ 0.176	17.15 $\pm$ 0.217	+0.158 $\pm$ 0.017	+0.229 $\pm$ 0.016

\*The budlings and orchard trees on sour stock were in each case 2 years younger than those on sweet stocks.

It will also be seen from a study of table 12 that there is a rather marked degree of difference in the coefficient of variability of the budling trunks; for the population on sour stock it is 25.43 per cent, while for the sweet stock it is 19.13 per cent. This difference is readily understood when it is remembered that the budlings on sour stock were measured at the end of 1 year after the buds were inserted, while those on the sweet stock were not measured until 3 years after the budding. As observed above, the coefficient of variation of young budlings, which is at first very high, normally decreases very rapidly through several years.

It will be noticed also that the coefficient of variability in the yield of the orchard trees on sour orange (34.11 per cent), is about double that of the trees on sweet stock (17.15 per cent). This difference in range of variability is understandable in view of the fact that the yields on the sour-stock trees were for a shorter period and from younger trees, where a higher range of variability is to be expected.

## RESULTS FROM PRACTICAL ORCHARD EXPERIMENTS

Evidence from field experiments is usually somewhat faulty because it is limited to small populations, and supplementary information obtained from orchard plantings may sometimes be of very great value. Such evidence bearing on the problem under consideration was furnished by a Valencia orange grove of 60 acres on sour-orange stocks on the San Marino ranch near Pasadena.<sup>14</sup> In the planting of this grove the nursery trees used were grown and budded on the ranch, and many more trees were grown than were required for the prospective planting. All were budded from selected trees on the ranch.

It was at first intended to plant only 20 acres with these trees, and the largest and best trees in the nursery were chosen and used for this planting. Since many good trees remained after the planting of the first 20 acres, it was decided to plant a second 20 acres, and the nursery was subjected to a second selection of the best trees remaining. These were planted in an orchard adjoining that made with the trees of the first selection. As a number of small and apparently healthy trees still remained in the nursery, a third tract of about the same size was planted with them.

These trees were thus planted in three sections of the same orchard at slightly different times, but they were all on seedlings of the same age, grown in the same nursery, and budded at the same time with buds from the same source.

The writer made a careful study of the three sections of this grove in 1922 when the trees were 5 years old. That portion of the grove planted with the largest nursery trees (first selection) had made a fine growth. The trees were remarkably uniform in size, and were large, vigorous, and fruitful, as shown by the mature crop on the trees at the time the examination was made and the young fruits set for the next year's crop.

The portion of the grove planted with intermediate-sized trees (second selection) had made a fair growth, but the trees were much smaller and less fruitful than those in that part of the orchard planted with large trees. While this portion of the orchard was fairly uni-

<sup>14</sup> The details relative to the planting of this orchard and the results obtained were described by the writer in an earlier paper (Webber, 1922). Unfortunately this grove was destroyed when 8 years of age to make room for city development.

form, it contained a considerable number of undersized trees. Very few of the largest trees were as large as the smallest in the part of the orchard planted with large trees.

The portion of the orchard planted with the small trees was highly variable in tree size and growth and was much inferior in size and yield to either of the other two plantings.

In 1924 when the trees in this orchard were 7 years old, a study of the three sections was made by Mr. Glenn C. Nay, a graduate student of the University of California, working under the writer's direction. Comparable blocks containing 100 trees each were chosen in each section and measurements made of height of tree, diameter of top, circumference of scion trunk, and circumference of stock trunk. A summary of these data is given in table 13.

TABLE 13

AVERAGE MEASUREMENTS OF 7-YEAR-OLD TREES IN BLOCKS PLANTED WITH LARGE, MEDIUM, AND SMALL BUDLINGS; SAN MARINO RANCH, PASADENA, CALIFORNIA

Grade of budlings	Population	Average height of tree	Average diameter of tree top	Average circumference of scion trunk	Average circumference of stock trunk
		<i>feet</i>	<i>feet</i>	<i>inches</i>	<i>inches</i>
Large .....	100	8.8	8.7	14.2	15.1
Medium .....	100	7.7	6.8	10.9	11.4
Small .....	100	6.7	5.9	10.4	11.0

It does not seem probable that the variation in soil, buds, or bud unions could be responsible for the differences exhibited by these three sections of the same grove. It is difficult to escape the conclusion that they were primarily due to the differences in the size of the nursery stock selected for the planting. Since uniformly good buds were used it seems likely that the most important variable was concerned with size and type of the rootstock seedlings.

Several other orchards have been examined that furnish similar suggestions, but, as the data is in agreement with the one cited and with the results of the experimental plantings herein discussed, they will not be described here.

## GENERAL DISCUSSION OF RESULTS

Ever since the early beginnings of agriculture, propagation from the best individuals has been more or less generally practiced. This practice and general understanding was crystallized by Darwin into the principle of "improvement by selection." Theories concerning the type of variation on which selection acts, have changed greatly in recent years; but the fact that selection of the best individuals as parents for propagation maintains or improves a race or breed, has not been and probably cannot be, questioned. No scientific principle on which agricultural practices are based would seem to be more surely established than this.

The selection of rootstocks, however, has seldom been practiced further than to choose the type or species giving the best results generally. The results presented in this study do not approach the question of what type or species is the best stock for a certain fruit variety on a certain soil, but furnish evidence relative to the importance of selecting the best individuals for stocks within the species or variety.

### SIZE AS A MEASURE OF SUPERIORITY

The question in considerable measure hinges on what constitutes the best individuals. Are the largest and most vigorously growing seedlings to be considered the best or might not smaller, more slow-growing types prove superior? It might well be that some stock type normally of slower growth or one of more rapid, vigorous growth would prove superior to those now used. The results, however, do tend to prove that whatever type is used, the normal, vigorous individuals of that type should be chosen and the weaklings and variants discarded.

Throughout the discussion, emphasis has been placed on the importance of large size of tree, and yet the question might be raised as to whether large size of tree is correlated with high yield, and whether after all, large size of tree is important. This emphasis seems to be warranted since in the present study, size of tree has been found to be positively correlated with yield. It will be remembered that comparisons between trunk area and yield in the years 1927 and 1929 gave correlations of  $+0.257 \pm 0.034$  and  $+0.261 \pm 0.033$ , respectively. Parker and Batchelor (1932) also obtained similar correlations between size and yield during five different years. These



correlations, as Parker and Batchelor point out, are positive and significant and it seems that we may safely conclude that under ordinary conditions where citrus trees of the same age have been treated similarly, the largest trees will most commonly be the highest producers.

In considering different varieties on different stock species, however, a different size standard would doubtless be required. If one were using a dwarfing stock in order to insure the production of trees below standard size it might be considered that the largest stock seedlings and the largest budlings should be discarded in order to insure dwarfing. The writer believes that this would be an erroneous policy; that whatever type of stock is used variants would be produced that would give still smaller trees—trees so weak that they would be unsatisfactory; and thus that even when a dwarfing stock is chosen, the strongest and best individuals of this stock should be used.

#### THE BASIS OF CITRUS ROOTSTOCK SELECTION

*Evidence Indicating Effectiveness of Selection.*—As pointed out in the introduction of this paper, the importance of selection within the type in connection with rootstocks used for propagation had been disregarded and apparently largely overlooked prior to the appearance of the writer's first publications on this subject (1920 and 1920a). The first experiment in testing the results obtained by choosing large, medium, and small budlings from the same batch of nursery trees indicated in general that the various sizes tended in considerable degree to retain the same relative size throughout a period of 12 years. Out of the 9 plots of three varieties, only 1 plot, that of small Valencias, proved an exception.

In this experiment the correlations of nursery budling size with trunk area and top volume of 12-year-old orchard trees were positive and fairly large, for all three varieties (table 2). Similar correlations between size of nursery budlings and total 6-year yields were for Marsh grapefruit moderately large ( $+0.410 \pm 0.085$ ), for Washington Navel small, and for the Valencia orange, due to the exception in the plot of small trees, slightly negative.

In the data presented from a population of 389 trees in a second experiment, the correlation between the size of nursery seedlings at the time of budding and the size of the budlings when 1 year old, was positive and large (table 3). The correlation coefficients between the size of 1-year-old nursery budlings as indicated by area of trunk

cross section, and the trunk area, top volume, and 5-year yields per tree of the 8-year-old orchard trees grown from them in the same experiment, were all positive and sufficiently large to be very significant (tables 6, 7, and 8). It is evident, therefore, that with citrus a severe selection based on size should be made, either of the stock seedlings or of the budlings before they are transplanted to the orchard or possibly of both. The determination of the best method of selection to pursue, however, is complicated by the presence of variant types among the stock seedlings.

The number of these variant types present differs mainly in accordance with the percentage of sexually produced embryos developed by the particular stock type used. In ordinary lots of sour or sweet-orange seedlings they would probably amount to from 15 to 25 per cent of the total number of seedlings produced.

The seed-bed stock in this experiment, after discarding the smallest seedlings to the extent of about 10 per cent of the total population was graded into first grade (large) and second grade (small). At the time of the critical study and numbering of these seedlings, just before they were budded, as described on page 24, it was found that much the largest number of variants were among the seedlings classed as second grade. By the discarding of all of the second-grade seedlings, 81.18 per cent of all of the evident variants would have been thrown out, but 18.82 per cent of the variants would still have remained among the lot graded as firsts, and these could only be detected after the seedlings had been grown a year or two in the nursery.

The problem of improving nursery stock depends primarily on the elimination of these variant types so far as they can be recognized, and, apparently, secondarily on the elimination of a certain percentage of the remaining seedlings that are below average vigor.

In a preceding section of this paper it was pointed out that in the seed bed, under the severe crowding that occurs as seed beds are ordinarily grown, the size of the seedlings of normal type apparently cannot be taken as an indication of their growth rate and character. Their size is probably due in large measure merely to the incident of the varying size of the embryo, promptness or delay of germination, and their location in the seed bed. Thus if all of the small seedlings up to from 45 to 50 per cent of the total population (the second-grade seedlings, paragraph above) are eliminated at the seed bed, perhaps 80 to 85 per cent of all the variants would be removed; but a considerable number of good stock seedlings would also be discarded.

Meanwhile from 15 to 20 per cent of the variants would remain with the selected large seedlings, and these can only be detected later in the nursery where they are given sufficient space to develop and exhibit their characteristics.

It seems evident, therefore, that a single selection at the time of digging the seed bed, sufficiently severe to eliminate the greater part of the variants, would be impractical, as some variants would not be detected and eliminated, and the destruction of good seedlings would be too great. The elimination should probably not be greater than 25 to 35 per cent of the total number of seedlings; this will take out all of the seedlings so small as to be difficult to transplant and also a considerable proportion of the variants.

The further selection to eliminate the variants apparently should then be made in the nursery just before the budding begins. At this time, if the seedlings have been allowed to grow until they have reached a diameter averaging about  $\frac{3}{8}$  inch, which when 1-year-old seed bed stock is used, will usually require 2 years (or 2 growing seasons) in the nursery, they will have had sufficient time to exhibit more fully their true characters. The small and variant types can be detected at this time and not budded. Such roguing should also eliminate the individuals of normal type that are too weak to produce good trees.

The examination and selection at this period is probably to be considered as the most critical and important; for it is the only period when the top characters of the stock seedlings can be seen. It is also done before the expense of budding has been added to the value of the tree. Attention should be directed to the fact that nurseries throughout California are now very generally budding younger and smaller seedlings than those used in this experiment or than were commonly used by nurserymen a few years ago. The variations in size and type in the younger seedlings cannot be so easily recognized and the elimination of variants is thus more difficult.

When the small seedlings have been removed at the seed bed and all evident variants have been eliminated in the nursery, the data from two different experimental populations, one on sour stocks and one on sweet stocks, indicate that small positive correlations exist between size of budlings, as shown by area of trunk cross section, and size and yield of 8 and 10-year-old orchard trees (tables 6, 7, 8, and 11). These rather small correlations may leave one in doubt as to whether any further selection would be effective.

It must, furthermore, be remembered that in comparing the size of seedlings at the time of budding with the size of budlings and of orchard trees at various ages when the variants were eliminated, the correlation, which with 1-year budlings was fairly large, gradually decreased to nothing with 8-year-old trees (table 4). The size of the stock trunk at time of budding correlated with size of stock trunk and also with top volume of the orchard trees after 8 years gave small but not significant negative correlations.

With yield, however, the case may be somewhat different. The area of seedling stock trunk at time of budding correlated with total 5-year yield gave a coefficient of  $+0.135 \pm 0.035$ . This is a positive correlation and though it is small, it may be of some significance (see discussion on page 39). This would seem to be the case in view of the result obtained by a practical selection of the trees based on seedling size in the nursery as described later in this section.

In general, however, it would seem from the fact that no positive correlation was obtained when seedling trunk area in the nursery was compared with scion trunk area and volume of top of orchard trees, that if any selection is to be made further than that designed to eliminate the variants, it should be based on budling size in the nursery. This is in view of the fact that there is a small but significant positive correlation of the scion trunk area of the budlings with all characters measured in the orchard trees, namely, size of trunk, volume of top, and yield.

*Results Obtained by Selection of Seedlings.*—In order to obtain more direct information as to the value of any selection other than the elimination of the variants, it was decided to make an actual selection based on size of seedlings just before they were budded, and on 1-year-old budlings, and to determine the results produced. The only population on which the data necessary for testing such a selection is now available is that from the experiment described on pages 23 to 53 of this paper. This population after the elimination of the small seedlings at the seed bed and of all variants, contains 346 trees on which full records have been made. A selection can thus be made of these trees on the basis of the diameter of the seedling rootstocks just before the budding. A segregation of the trees was thus made into three classes, namely, first or large grade, containing all trees of which the seedling trunks at time of budding had a diameter of 2.2 cm or over; second or medium grade, containing all trees with seedling trunk diameters of 2.1 cm (this is the modal class); and third or small grade, containing all trees with seedling

trunk diameters of 2.0 cm or less.<sup>15</sup> This segregation gave 109 trees, or 31.5 per cent, in the small or third grade; 73 trees, or 21.1 per cent, in the medium or second grade; and 164 trees, or 47.4 per cent, in the large or first grade.

The yields for the 5-year period during the time when the orchard trees were 4 to 8 years of age, were segregated into the above three classes, and the total 5-year yield and average yield per tree determined for each class. These data are brought together in table 14.

TABLE 14

COMPARATIVE YIELDS OBTAINED BY SEGREGATING STOCK SEEDLINGS (AFTER ELIMINATION OF VARIANTS) INTO LARGE, MEDIUM, AND SMALL GRADES\*

Grade	Diameter of seedling trunk	Number of trees	Per cent of total population	Total average yield per tree	Gain in yield per tree over third grade		Value of gain per tree at 2 cents per pound†
					pounds	per cent	dollars
Third (small)	cm 2 or less	109	per cent 31.5	pounds 238.2			
Second (medium)	2 1	73	21.1	291.5	53.39	22.4	1.07
First (large)	2 2 or over	164	47.4	295.6	57.49	24.1	1.15
Firsts and seconds combined	2 1 or over	237	68.5	294.4	56.22	23.6	1.12

\* Total population 346 mean total yield for 5-year period, 277.18 pounds per tree

† Over 5-year period

It will be seen from an examination of table 14 that the third-grade trees gave an average yield during the 5-year period of 238.2 pounds per tree; while for the same period, the second-grade trees gave an average yield of 291.5 pounds; and the first-grade trees an average yield of 295.6 pounds per tree. It will be seen that the average yields per tree of the first and second-grade trees do not differ greatly, but that the yield of the third-grade trees is considerably lower. The gain in average yield per tree over the third-grade trees was for the firsts, 57.5 pounds; and for the seconds, 53.4 pounds. In view of the great variation in yields due to environmental and other causes the small difference between the gain found for the firsts and seconds could probably not be considered significant, and any selection discarding the seedlings placed in the second or medium class would probably be meaningless. If the first and second grades are placed together the combined population is 237, which is 68.5

<sup>15</sup> All measurements were made in centimeters and tenths of centimeters, and the segregations made here do not correspond to segregations into class intervals.

per cent of the total population concerned. This combined population would have an average yield of 294.4 pounds per tree, a gain over the third grade of 56.2 pounds per tree.

Is this gain in average yield per tree sufficiently great to justify a further elimination after the variants are excluded? The elimination of the third-grade seedlings from this population would have meant the elimination of 31.5 per cent of the total number. By sacrificing this proportion of the seedlings there is obtained a gain in average yield per tree of 56.2 pounds, which at 2 cents a pound means a gain of \$1.12 a tree in value of fruit produced during the early 5-year period of fruiting. This seems sufficient to justify the payment of a fairly high price per tree to insure at least this degree of selection. The value of good seedlings before budding is comparatively small, certainly under normal conditions not over 15 to 20 cents each.

This, perhaps, is a rather confusing result in view of the lack of correlation between seedling size and size of 8-year-old orchard trees, but it should be remembered that there was a small correlation,  $+0.135 \pm 0.035$ , between area of seedling trunk and total 5-year yield, which ordinarily would not be considered significant.

*Results Obtained by Selection of Budlings.*—It is important in determining the most practical method of selection to question whether the main selection might not better be made at the time when the budlings are being dug from the nursery for transplanting, rather than as seedlings before budding, since a few buds possibly will heal poorly. In order to test this idea, the same population of 346 trees after the elimination of the variants, was subjected to a selection based on the size of the 1-year-old budlings as indicated by trunk diameter 2 inches above the bud union. Here, as in the preceding case, a segregation of the trees was made into three classes, namely, large or first-grade trees, containing all trees the scion trunks of which at 1 year of age had a diameter of 1.9 cm or more; medium or second grade, containing all trees with a scion diameter of 1.8 cm, the modal class; and small or third grade, containing all trees with a scion diameter of 1.7 cm or less. This segregation gave 104 trees, or 30.1 per cent, in the small or third grade; 69 trees, or 19.9 per cent, in the medium or second grade; and 173 trees, or 50.0 per cent, in the large or first grade.

The total yields per tree for the 5-year period, when the orchard trees were 4 to 8 years of age, were segregated according to these

three classes and the total 5-year yield and average yield per tree determined for each class. These data are brought together in table 15.

TABLE 15

COMPARISON OF YIELDS OBTAINED BY SEGREGATING BUDLINGS (AFTER ELIMINATION OF THOSE ON VARIANT SEEDLING STOCKS) INTO LARGE, MEDIUM, AND SMALL GRADES\*

Grade	Diameter of scion trunk	Number of trees	Per cent of total population	Average yield per tree	Gain in yield per tree over third grade		Value of gain per tree at 2 cents per pound†
					pounds	per cent	dollars
Third (small) .....	cm 1.7 or less	104	per cent 30.1	pounds 243.7			
Second (medium) .....	1.8	69	19.9	270.0	26.35	10.8	0.53
First (large) .....	1.9 or over	173	50.0	299.1	55.47	22.8	1.11
Firsts and seconds combined	1.8 or over	242	69.9	290.8	47.17	19.4	0.94

\* Total population 346; mean total yield for 5-year period, 277.18 pounds per tree.

† Over 5-year period.

*A Comparison of Seedling and Budling Selection.*—It will be seen from an examination of table 15 and a comparison of the data with those in table 14 that the budling selection is apparently slightly inferior in results to that obtained by the seedling selection. In the third-grade class of small trees presumably to be discarded there are by the budling selection 104 trees with an average yield of 243.7 pounds per tree against 109 with an average yield of only 238.2 pounds per tree when based on a seedling selection. The seedling selection has thus eliminated 5 more trees and the average yield of the whole lot is 5.5 pounds per tree less than the average yield of the 104 trees discarded by the budling selection. The average yield of the second-grade trees for the budling selection is 270.0 pounds per tree as compared to 291.5 for those of the seedling selection of the same grade. For the first-grade trees of the budling selection, however, the yield is 299.1 pounds per tree in comparison with 295.6 for the first-grade large trees of the seedling selection.

If one is to make a very severe selection and weed out both small and medium-sized trees, the budling selection would give a group of trees much reduced in number but with a higher average 5-year yield than those of the same grade of the seedling selection. Even with this severe selection, however, the gain in yield of the first-grade budlings over that of the third-grade budlings is smaller than the gain between the corresponding grades in the seedling selection. If only the first-grade trees of the budling selection were preserved,

there would be discarded 173 budded trees of salable age. This severe selection would be very expensive.

The careful consideration of these figures and the entire data, it seems to the writer, points rather strongly to the conclusion that with this population a selection of the seedlings just preceding the budding to eliminate approximately 109 of the smallest, or about 31.5 per cent of the total number, was the better method of selection to pursue, and greatly reduced the cost involved in making the selection.

The following further notes on this selection will serve to strengthen this conclusion. The total 5-year yields of these trees varied from 14 pounds per tree to 484 pounds per tree. Choosing arbitrarily 200 pounds per tree as a fair yield and examining the data, the following results are obtained:

1. Good plants, yielding 200 pounds or over, that would be lost by seedling selection but saved by budding selection—26.
2. Good plants, yielding 200 pounds or over, that would be lost by budding selection but saved by seedling selection—28.
3. Poor plants, yielding less than 200 pounds, that would be retained by seedling selection but eliminated by budding selection—8.
4. Poor plants, yielding less than 200 pounds, that would be retained by budding selection but eliminated by seedling selection—17.

It will be clearly recognized that data obtained from a population of 346 trees cannot be considered as conclusive, but this number of trees carefully handled and accurately graded should furnish valuable suggestions. Data have been presented from a similar and much larger population of 1,506 trees, with variants excluded, showing that the correlation of size of budding with size and yield of orchard trees was practically the same as with this population of 346 trees.

It will be remembered that the comparison of seedling size in the nursery with the size of 8-year-old trees gave a very small but not significant negative correlation, indicating that no consistent and sustained relation exists; and yet in carrying out the selection based on seedling size, a fairly satisfactory result was obtained which was rather better than a similar selection based on size of budlings where a small positive correlation was shown to exist regularly. There was, however, a strong correlation between seedling size and budding size, and a small positive correlation between seedling size and yield during the first 5-year period. It is, therefore, evident that the benefit derived from discarding the small seedlings is maintained sufficiently long to result in a financial return that justifies the selection.



If the policy suggested by the results just outlined is followed, and the main selection in the nursery is made just previous to budding, then the further question is suggested as to whether any selection of the budlings is desirable. Such a selection would naturally be made at the time of digging the budlings and would most naturally be based on the size of the budling trunk. The population of 346 trees can be subjected to this further experimental selection. The selection of the seedlings in the nursery just before budding eliminated 109 seedlings of the third grade and there thus remains a total of 237 budlings ready to transplant to the orchard. If the same basis of segregation as that in table 14 is now used with these 237 budlings, placing in a third grade for discarding all of those with a diameter of scion trunk of 1.7 cm or less, 39 budlings would be discarded, and the recorded total 5-year yields of these show that 18 of them were below the mean yield of the modal class (260 to 279 pounds), and 19 above the modal class, with 2 in the modal class. The average 5-year yield of the 39 trees that would be discarded is 261.3 pounds, which is only slightly below the average for the whole population exclusive of variants. A careful examination of the whole history of these 39 trees does not indicate any means by which the few low-yielding trees could have been segregated from the good ones. All excepting possibly 2 or 3, are now of comparatively normal size and their low yields are probably due to environmental causes. It would seem, therefore, that it may be safely concluded that after a selection of seedlings as indicated, the only selection to be exercised among the budlings before they are dug from the nursery should be designed to eliminate the few very smallest ones that have not grown well and are too small to transplant safely.

The case relative to methods of nursery selection as indicated by these studies may be summarized as follows:

1. The results obtained have clearly demonstrated the importance of eliminating all variant types from among the seedlings to be used as rootstocks. The selection to accomplish this, judging from the results obtained, probably should be made both by discarding the small seedlings at the seed bed, and the small and off-type, variant seedlings in the nursery just before budding.

2. It would seem that after the elimination of the variants, the seedlings should be subjected to a further selection to eliminate the small ones to the extent of possibly one-third of the total number remaining. This is based on the fact that the experimental population after the elimination of the variants gave a small, but possibly

significant, correlation between seedling size and total 5-year yield and also that the elimination of the small seedlings from the population remaining after the variants were discarded resulted in an increased average yield for the first five years of bearing.

In view of the fact that the elimination of the small seedlings and budlings cannot be injurious even if no permanent improvement is achieved, and in view of the fact that the gain in yield during the first few years will probably pay for the cost of the selection, it seems evident that the safest and best policy to pursue is to practice a severe nursery selection. This will insure the elimination of the variants and probably will give a better yield for the first few years. It will probably also insure more uniform trees and a better yield during the whole life of the orchard.

*The Influence of Selection Within Apogamic Progenies.*—Under "Variation, Apogamy, and Polycembryony in Citrus" (pages 6–15), the types of variation with which this study is concerned were discussed in some detail, as was also the relation of apogamy to the problem of securing citrus stocks of uniform genetic constitution. In the experimental populations studied it has been shown that the most important step in the methods of selection suggested is the elimination of the variants. It has also been shown that the seedlings remaining after the variants are excluded, may be considered to be almost wholly of apogamic origin and thus, if the seeds came from the same mother parent or clon, to possess the same genetic constitution.

In view of the investigations of Johaussen and many others on selection within pure lines, where apparently no significant advance has been obtained, unless there occurred a change of type (mutation), selection within a genetically homogeneous apogamic progeny might be thought to hold little promise.

The investigations on the effectiveness of selection within pure lines, however, so far as the writer is informed, have been directed wholly toward the discovery of whether a change could be induced which would have permanent genetic significance. The investigations, furthermore, have all been with very short-lived plants and animals and never with long-lived perennial trees. It would seem to be entirely reasonable to assume that the selection of the largest seedlings within an exclusively apogamic population or of the largest nursery plants within a single clon might result in a distinct improvement in the size of tree and quantity of the crop produced during a part or all of the life of the trees, and yet not indicate any change of the type that could be considered of genetic significance.

The evidence from the experiments described in this paper indicates that when dealing with populations from which all variants that could be detected had been eliminated and which were thus supposedly of nearly pure apogamic origin, there was still a considerable range of variation exhibited as indicated by area of cross section of trunk, volume of top, and average yield. The evidence seems also to indicate rather strongly that the elimination from the homogeneous apogamic population in the nursery of the smaller seedlings and smaller budlings tends to increase the size and yield of the orchard trees propagated on those remaining. The increase in yield during the first 8 years from such selected trees in one experiment was found to be sufficient to cover the expense involved in making the selection.

There is thus apparently an advantage possessed by the seedlings and budlings that are large while growing in the nursery which is carried over when they are transplanted into the orchard and persists for a considerable period, at least long enough to give a larger crop of fruit during the first 8 years. There is no evidence available to indicate definitely how long this superiority of the population propagated on the selected stocks will be maintained.

If a selection in the nursery to eliminate the small seedlings and budlings is effective and of practical value in an apogamic progeny where the genetic constitution of the mother is maintained, it should be equally effective with stocks reproduced by cuttings, slips, or layers. Evidence confirming this is furnished by the results obtained by Bioletti (1926) in an experiment testing the effect of size and quality of rootings (rooted cuttings) of the grape. The influence was very marked during the first three seasons in the vineyard, was noticeable in the fourth year, but had largely disappeared by the end of the fifth year. He states:

The first crop of the vines [during the third season] from the strongest 25 per cent of the rootings was about 50 per cent larger than the first crop of the vines from the weakest 25 per cent. This difference was in great part reversed by the second crop and there was little difference in the third crop.

The advantage of the strongest rootings was in reaching nearly full bearing the third season instead of the fourth as with the weaker rootings. The poorest rootings (used in this experiment) were all equal to what are usually considered No. 1 quality. With more imperfect rootings such as are very commonly planted, the difference would undoubtedly have been greater.

Although the evidence with citrus trees presented in this paper is not sufficient to justify a final conclusion, it seems to indicate that with perennial fruit trees an improvement will result from a selection, among an apogamic or clonal progeny, of stock plants of superior

size and vigor. This improvement can probably be explained as due to the long hold-over influence of a more favorable start, which influences the plant during a considerable portion of its life cycle, and not to any change of genetic nature caused or stimulated by the selection.

Apparently the general principle involved here is the same as that concerned in the production of larger yields by the use of large seeds after an elimination of the small ones. Markedly increased yields obtained by such seed segregations have been reported for almost all annual crops propagated by seeds.

Blackman (1919) has pointed out that the stored nutrition carried by the seed or plant body may be considered as the capital investment with which the young plant starts growth and that the growth rate of the plant may be likened to the interest rate which is compounded very frequently. If the growth rate (interest) is the same in two plants, that one starting with the largest quantity of stored material (capital) may be expected to remain the largest if grown under uniform conditions.

With perennial trees having the same growth rate and grown under uniform conditions those that are largest in the beginning should theoretically remain the largest until the trees approach mature age and size when growth rate is equalled by decay.

## METHOD OF NURSERY SELECTION SUGGESTED

Based on the data and results outlined in this paper the following methods of nursery selection are suggested:

*Seed Bed Selection.*—When the seedlings are dug from the seed bed discard the smallest seedlings to the extent of about one-fourth of the whole population, or 25 per cent. Discard at this time all malformed seedlings such as extreme cases of “goose neck” or “bench root.”

Twenty-five per cent is an arbitrarily chosen quantity. This is, however, rather more severe than the ordinary selection made at the seed bed and will eliminate a considerable proportion of the variant types.

*Nursery Selection.*—When the seedlings growing in the nursery have reached the size and age for budding, go over them carefully and cut out all plants differing sufficiently from the standard or normal type of the stock to be recognized as distinct and peculiar in any character. This elimination should be irrespective of size. After this elimination of variants, the small seedlings should be cut out and dis-

carded to the extent of from 25 to 30 per cent of the entire remaining population.

After this elimination has been made the entire remaining population may be budded and safely considered as propagated on good, uniform, highly selected stocks. As the seedlings up to this time, previous to budding, have little value, the carrying out of this elimination does not entail very great financial loss.

*Budding Selection.*—Some buds do not heal well or are defective and do not give vigorous good trees. Such small and inferior buddings should be cut out and discarded before the buddings are dug for orchard planting. With good buds and selected large stocks the elimination at this time will be very slight, probably not over 1 to 5 per cent.

## SUMMARY

The investigations discussed in this paper are concerned with: first, the influence that variable seedlings used as rootstocks exercise on the size and yield of orchard trees; and second, the determination, if possible, of a basis of selection that may be used in the improvement of citrus nursery stock.

Citrus seedlings, of the species and varieties most commonly used for rootstocks, exhibit a wide range of variation. In any lot of seedlings grown from seed of the same variety and from the same source, the great majority usually are of the same general type, but from 5 to 40 per cent of them are highly variable types which apparently differ in genetic constitution from the prevailing type and from each other.

The evidence available indicates that the seedlings of the prevailing type originate from apogamic embryos and are thus, presumably, of the same genetic constitution as the seed parent or parents. Some citrus varieties exhibit a very high degree of apogamy (80 to 100 per cent), and by the use of seeds from such sorts, large lots of seedlings almost uniform genetically and of the same type can be easily obtained for use as stocks.

The seedlings of variable types which are present in small numbers in all lots of citrus seedlings (except those of sorts which are 100 per cent apogamic) are here termed *variants*. They are probably developed from the normally produced sexual embryos mainly by self-fertilization, but to some extent by cross-fertilization. The seedlings of variant types are usually small and lacking in vigor, and when used as stocks

are found almost invariably to produce orchard trees exhibiting some degree of dwarfing.

In one experiment, large, medium, and small budlings (nursery trees) of Washington Navel and Valencia oranges and of Marsh grapefruit, all propagated on sweet-orange stocks, were grown side by side for comparison. After 11 years in the orchard under approximately uniform conditions, the correlations of budling trunk area with the trunk area, volume of top, and total 6-year yields of the orchard trees in all but 1 of the 9 plots were positive and significant.

The following results were obtained from a study of another carefully planned experiment with 389 trees of Washington Navel orange on sour-orange stocks. Each tree was observed from the seed-bed stage through the nursery and for 8 years after planting in an experimental orchard.

1. The seedlings, when dug from the nursery, and after the elimination of the very small ones, were segregated into two grades, large and small, which were kept separate in the nursery. Among these there were 43 seedlings of variant types which later were found to cause marked dwarfing of the orchard trees budded on them and were very unsatisfactory. Seventy-seven per cent of these variant types were among the seedlings graded as small at the seed bed.

2. These variant seedling types were propagated on sour-orange and Rough-lemon stocks, one tree on each stock, and were found to maintain their marked varietal characteristics unchanged by the influence of the two stocks. The stock influence was limited apparently to quantitative characters only, such as differences in size.

3. The size of nursery seedlings, as shown by area of trunk cross section correlated with the size of budlings and 8-year-old orchard trees, gave coefficients respectively of  $+0.736 \pm 0.016$ , and  $+0.437 \pm 0.028$ . These fairly strong correlations were influenced to a considerable extent by variants, as a result of their dwarfing influence.

4. When the population, exclusive of variants, was used and size of seedlings compared with the size of budlings and with 2, 6, and 8-year-old orchard trees, the correlations were  $+0.549 \pm 0.026$ ,  $+0.125 \pm 0.036$ ,  $+0.010 \pm 0.036$ , and  $-0.021 \pm 0.037$  respectively, indicating that there was a temporary relation between the size of selected seedlings and the size of trees after planting in the orchard.

5. The size of nursery seedlings, exclusive of variants, compared with the top volume of 8-year-old trees gave no correlation ( $-0.012 \pm 0.036$ ), but it is possibly significant that with a total 5-year yield per tree a positive correlation of  $+0.135 \pm 0.035$  was obtained.

6. The size of 1-year-old budlings compared with the size of 8-year-old orchard trees gave a correlation of  $+ 0.622 \pm 0.021$  for the entire population; and for the population exclusive of variants at 2, 6, and 8 years old gave correlations respectively of  $+ 0.358 \pm 0.032$ ,  $+ 0.170 \pm 0.036$ , and  $+ 0.182 \pm 0.034$ .

7. Size of budlings compared with 5-year yields gave for the entire population a correlation of  $+ 0.517 \pm 0.025$ , and for the same population exclusive of variants  $+ 0.233 \pm 0.034$ . Thus there was a general tendency for the large budlings to produce large, high-yielding trees, and for the small budlings to produce small, low-yielding trees.

8. The coefficients of variability for size of tree gradually decreased as the trees grew older.

9. The comparison of trunk area of budlings with top volume of 8-year-old trees gave for the entire population a correlation of  $+ 0.598 \pm 0.022$  and for the population exclusive of variants,  $+ 0.202 \pm 0.035$ , thus corresponding very closely with the results obtained when the trunk areas of budlings and of 8-year-old trees are correlated, and also with the correlation of trunk area of budlings with 5-year yields.

10. The close similarity of the statistical constants obtained when different measures were used (such as area of trunk, volume of top, and yields of fruit) are interpreted as indicating the reliability of the results.

11. There was a very close relation between the size of the trunk and the size of the top at the same period of development, as shown by the high correlations between trunk area in 1929 and top volume in 1930, which was  $+ 0.923 \pm 0.006$  for the entire population; and  $+ 0.817 \pm 0.013$  for the population exclusive of variants.

12. While the budling trunk area for the entire population exclusive of variants compared with the trunk area of 8-year-old orchard trees gave a correlation of only  $+ 0.182 \pm 0.034$ , the budlings with 2-year-old orchard trees gave a correlation of  $+ 0.358 \pm 0.032$ ; 2-year trees with 6-year trees,  $+ 0.618 \pm 0.023$ ; and 6-year trees with 8-year trees,  $+ 0.781 \pm 0.014$ . These rapidly increasing degrees of correlation for interperiods are interpreted as being caused probably by the cumulative influence of variations in soils rather than by variations in the rootstocks.

With this population (containing, after the elimination of the variants, 346 trees supposedly of apogamic origin and thus of nearly uniform genetic constitution) a segregation of the seedlings on the

basis of the recorded diameter at the time of budding gave 31.5 per cent of 2.0 cm, or less in diameter, and 68.5 per cent of 2.1 cm, or more in diameter. The orchard trees grown on the large seedlings gave an average 5-year yield of 56.22 pounds per tree more than the trees on the seedlings of the small group, an average gain per tree of 23.6 per cent.

A similar segregation of the same population on the basis of the recorded diameter of the budlings at the time of transplanting gave 30.1 per cent budlings of 1.7 cm or less in diameter, and 69.9 per cent of 1.8 cm or more in diameter. The orchard trees grown from the large budlings gave an average 5-year yield of 47.17 pounds per tree more than the trees of the small budling group, an average gain per tree of 19.4 per cent. These results indicate that a selection of the seedlings is as effective as a selection of the budlings; and it is much less expensive.

In a population of 1,506 Washington Navel trees on sweet-orange stocks from which all small budlings (and thus probably all markedly variant rootstocks) had been eliminated, the correlations between trunk area of the young trees and trunk area and total yields of 9-year-old trees were respectively  $+0.158 \pm 0.017$ , and  $+0.229 \pm 0.016$ . There is a noticeable and significant similarity between these correlations from Washington Navels on sweet-orange stocks and those from Washington Navels on sour-orange stocks.

In a commercial orchard where nursery trees were segregated at the time of planting into groups of large, medium, and small trees and planted separately in adjoining parts of the same orchard, the groups retained their relative differences in size up to the age of 7 years.

The evidence from all experiments and observations indicates that in general with entire populations, small seedlings and small budlings tend to produce small, low-yielding orchard trees; and that large seedlings and large budlings tend to produce comparatively large, high-yielding orchard trees.

The evidence clearly indicates that the most important factor in the improvement of citrus nursery stock is the elimination of the variant seedlings that were found almost uniformly to produce weak and dwarfed orchard trees.

The segregation of the seedlings when they were dug from the seed bed into first and second grades (large and small), was found effective only to the extent that it served to isolate with the seconds, 81.18 per cent of the variants. This proportion of the variants,



therefore, could have been destroyed by discarding all of the seconds, which comprised 47.77 per cent of the total population transplanted from the seed bed. The normal seedlings among those chosen as seconds at the seed bed gave orchard trees as satisfactory as the normal trees of the first grade.

The experiment indicates that the elimination of the variants can be accomplished, probably with the least loss, by a moderate culling of the small seedlings at the seed bed, and by the careful roguing and destruction of all variants and small seedlings in the nursery just prior to the budding.

The evidence also indicates that a selection based on the size of the seedlings or of the budlings remaining after the elimination of the variants, would result in a small but valuable improvement. This improvement is probably due to the long hold-over influence of a more favorable and better start which affects the plant during a considerable portion of its life, and not to any change of genetic nature caused or stimulated by the selection.

A plan of nursery selection is suggested which is based on the results of these studies and is designed for use in commercial nurseries.

### ACKNOWLEDGMENTS

For assistance in taking and recording field notes in connection with these experiments the author wishes to express his thanks especially to Robert McBride, Assistant Superintendent of Cultivations (deceased), and to Ralph G. La Rue, Superintendent of Cultivations in the Citrus Experiment Station. Thanks are also due especially to H. F. Stevens and E. R. Parker, both of the Citrus Experiment Station Staff, for assistance and suggestions in connection with the statistical work involved. The statistical calculations have all been made by Mr. Stevens working under the writer's direction.

Much of the work on one of the major experiments was carried on for several years by J. T. Barrett and due acknowledgment was made under the discussion of that particular experiment.

The writer wishes also to express his appreciation of the helpful criticisms and suggestions made during the preparation of this manuscript by Professor R. D. Anthony of the Pennsylvania Agricultural Experiment Station, State College, Pennsylvania.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

Vol. 7

OCTOBER, 1932

No. 2

## VARIATION IN THE YIELDS OF FRUIT TREES IN RELATION TO THE PLANNING OF FUTURE EXPERIMENTS<sup>1,2</sup>

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### INTRODUCTION

The yields resulting from field trials have, in many cases, indicated the varying responses of plants to soil conditions which appear to be independent of the considerations of the trial. These normal fluctuations in yield constitute a source of experimental error to which all field trials are subject. They are of such importance that they must be taken into account in the planning of such experiments, as well as in the interpretation of the results.

In orchard trials such errors may be especially large. The great variation observed is due, in part, to the relatively large area of land involved in a single experiment, with the attendant possibilities of important changes in soil and topography. It is also due in some degree to the individuality of the trees. These two classes of factors ordinarily increase the observed variations greatly above those found in experiments with agronomic crops, for in the latter the use of a large number of plants in a single plot results in practical elimination of the effects of individual plant variation. In addition, the relatively small size of the plots permits them to be located on a small area of land. In the case of

<sup>1</sup> Received for publication December 28, 1931.

<sup>2</sup> Paper No. 256, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

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agronomic crops, significant correlations frequently exist between the yields of nearby plots.

Another very important source of error in the interpretation of the results of trials with trees is due to the long life of the plants. Since cultural treatments may have cumulative effects upon soils and trees, and since responses in various seasons may differ, it is obligatory that experiments be extended over a long period of time. Consequently, the same individual trees and plots are employed repeatedly in the experiment in the same manner. Any individuality of the material and of the soil finds expression year after year in about the same way. This results in correlations between observations in succeeding years. In the case of trials with annual crops, however, this effect is largely eliminated by the use of different plants each year, and in many experiments by rearrangements of the location of the treatments during various years of the experiment.

In determining the relative effects of different treatments in any field trial, the ideal would be to ascertain the effect of the various treatments under absolutely identical conditions. In orchard work, such a situation is obviously impossible. The only possibility is to try each treatment simultaneously on a portion of the orchard. What is desired, then, is to obtain for each treatment a sample of the orchard which adequately "represents" the mean yield and variability of the entire orchard. The difficulties, as well as the importance, of obtaining such a sample have been demonstrated for orchard crops by the results of Batchelor and Reed<sup>(4)</sup> in their studies of the variability of several orchards.

Many methods have been proposed for correcting and interpreting the results of agronomic trials where there is doubt as to whether the individual plots represent a fair sample of the field as a whole. Most of these suggestions have been made as a result of studies upon uniformly planted and treated fields, where the effects of variability of the plants and soils could be studied in various years. Such studies have indicated that the extent and nature of the variations which have been observed differ in different plantings, and that each field presents some special problems. The variability of trees emphasizes the importance of similar observations in experimental orchards. It appears that each orchard used for experimental purposes should be individually studied.

It is the purpose of this study to determine, in part, the nature of the variations that exist in an experimental orchard which has been maintained a number of years under conditions of uniform culture. The bearing which this may have upon the efficacy of certain methods of

interpreting the results of the trials to be made upon this orchard, in relation to the manner of laying out the experiment, will be touched upon. The plan of an experiment will also be presented, which, it is hoped, may throw some light upon the problems involved in field trials with orchard trees and upon methods of minimizing their seriousness.

## MATERIAL

As a result of the studies of Batchelor and Reed<sup>(4)</sup> upon the variability of fruit trees, it appeared to them that observations might profitably be made upon the variability of trees destined for experimental use, while they are under a condition of uniform treatment, and prior to the beginning of the experiment. Therefore, in accordance with this idea and their other findings, an orchard of Washington Navel oranges was planted in 1917 at the University of California Citrus Experiment Station at Riverside. The ultimate purpose was to install a series of fertilizer trials in this orchard. The orchard was maintained under conditions of uniform culture for a period of ten years. The results of certain studies made upon data obtained from it are reported here.

Only a brief review of the plan and history of the experimental orchard is necessary for an understanding of the present paper. A more detailed account of the plan and history of the orchard has been published elsewhere by Batchelor, Parker, and McBride.<sup>(5)</sup>

In order to increase the accuracy of future trials, every practical means was employed to make the planting as uniform as possible. Land was selected which had been used for dry-farming grain culture from the time it was first cleared in 1875 until 1917, when the trees were planted. No leveling or grading was ever done purposely on this land.

Particular attention was paid to the selection of trees for this planting. Seedling rootstocks of sweet orange (*Citrus sinensis*) were used which had been culled three times to eliminate nonvigorous and undesirable types. The trees to form the experimental rows were budded to the Washington Navel variety. The buds were carefully selected from productive trees whose performance records were known.

Eight Washington Navel orange trees in a single row constitute a plot. A Valencia orange tree was planted as a border tree at the upper end of each plot row and a grapefruit tree at the lower end. Each two adjacent test rows of Navel oranges are separated by a guard row of Valencia oranges and grapefruit, which alternate in the guard row. Forty per cent of the trees are, therefore, test trees. The planting distance is 20 feet in the row and 24 feet between rows. Each test and each



guard tree occupies 0.011 acre. Each plot treatment is extended to the middle of each adjoining guard row, and also 10 feet past the end guard trees so that the treated area for each plot is thus equal to that occupied by 20 trees, 9,600 square feet, or 0.22 acre. The 199 plots occupy 43.86 acres. The arrangement of the trees in the plots and guard rows is given

		Row Numbers										
Pipe Line For Block	7	1	2	3	4	5	6	7	8	9	10	11
	1	G	V	G	V	G	V	G	V	G	V	
	2	V	N	V	N	V	N	V	N	V	N	
	3	G	N	G	N	G	N	G	N	G		
	4	V	N	V	N	V	N	V	N	V		
	5	G	N	G	N	G	N	G	N	G		
	6	V	N	V	N	V	N	V	N	V		
	7	G	N	G	N	G	N	G	N	G		
	8	V	N	V	N	V	N	V	N	V		
	9	G	N	G	N	G	N	G	N	G		
Pipe Line For Block	L	10	V	G	V	G	V	G	V	G	V	
	1	G	V	G	V	G	V	G	V			
	2	V	N	V	N	V	N	V				
	3	G	N	G	N							
	4	V	N	V								
	5											

Fig. 1. Arrangement of trees in plot and guard rows. N = Washington Navel orange; V = Valencia orange; G = Marsh grapefruit.

in figure 1. In planting the trees, an effort was made to mix them so that trees from every section of the nursery should be planted at random in the orchard.

The plots were planted in 1917 in 10 blocks which are lettered from D to M inclusive. The blocks consist of 12 to 27 plots each. The plot rows are numbered with even numbers in each block while the guard rows are numbered with odd numbers. The arrangement of the blocks and plots is shown in figure 2.

The slope of the land averages 1.6 per cent, and is, on the whole, fairly uniform, as shown in figure 3.

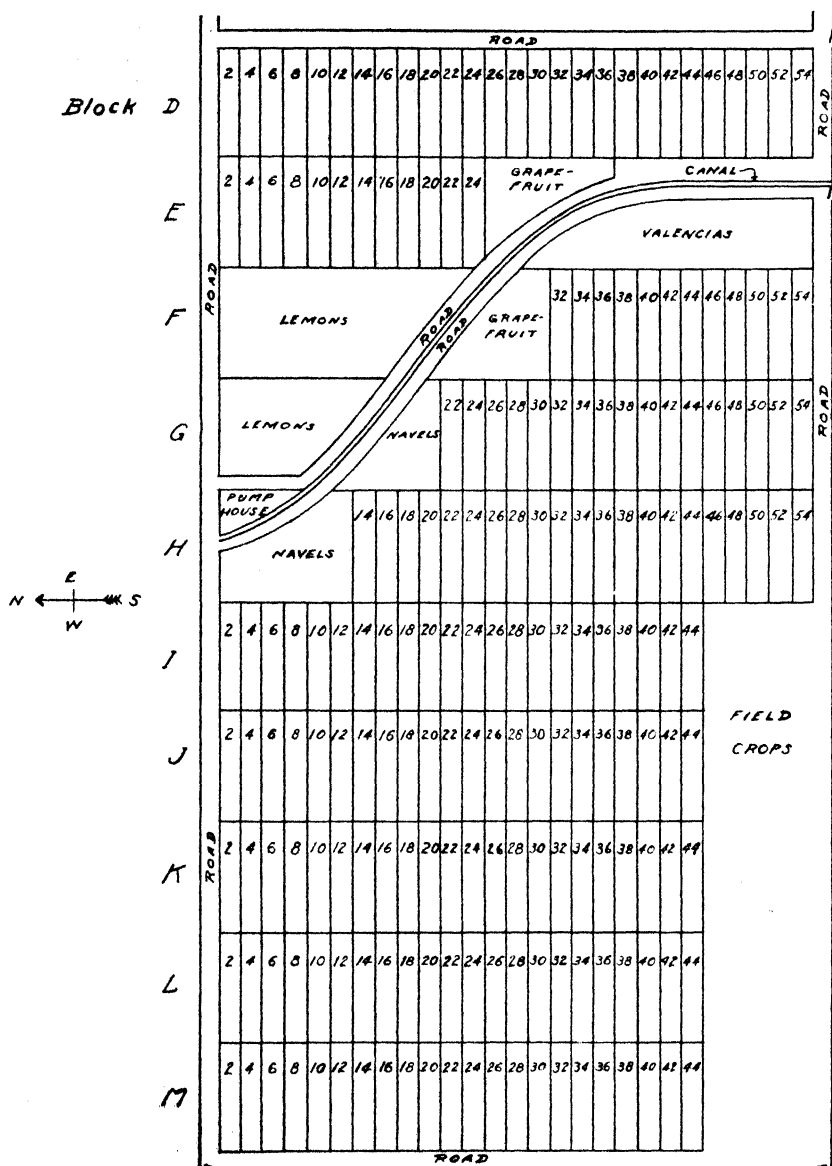


Fig. 2. Plan of experimental field showing arrangement of blocks and plots.  
(From Bul. 451.)

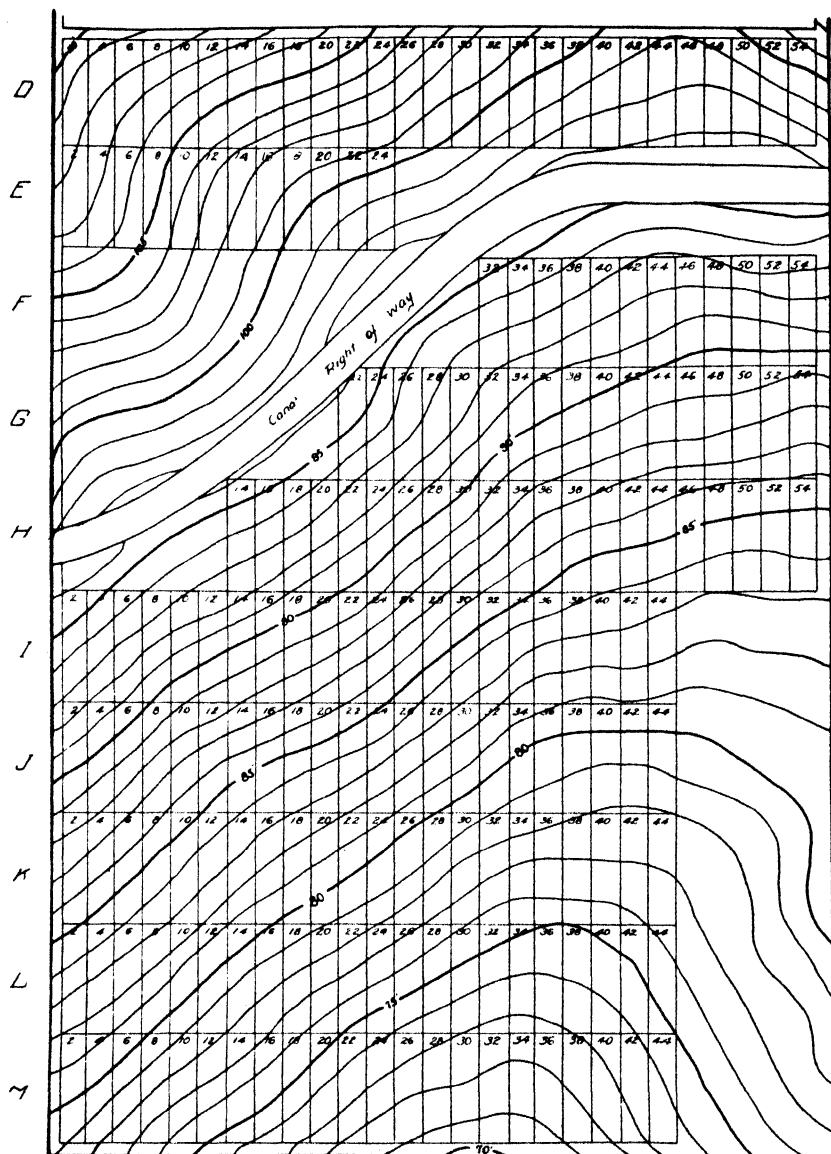


Fig. 3. Contour map of experimental area. Interval between contours equals 1 foot.

Irrigation systems have been installed so that each block is provided with a pipe line. This has made it possible to irrigate each block or even

TABLE 1  
FREQUENCY DISTRIBUTION OF YIELDS OF INDIVIDUAL TREES FOR EACH YEAR,  
1921 TO 1927\*

Yield in pounds	Number of trees, 1921	Yield in pounds	Year					
			1922	1923	1924	1925	1926	1927
			Number of trees					
0-4	226	0-9	3					
5-9	168	10-19	9	9				
10-14	171	20-29	24	28			2	1
15-19	143	30-39	33	70		1	3	1
20-24	157	40-49	102	113	1	1	10	1
25-29	196	50-59	155	211	0	4	12	2
30-34	217	60-69	151	248	4	7	24	2
35-39	67	70-79	127	264	5	8	54	9
40-44	114	80-89	363	199	13	29	102	15
45-49	31	90-99	165	156	36	49	118	22
50-54	13	100-109	136	112	55	81	156	19
55-59	5	110-119	126	64	63	146	175	39
60-64	1	120-129	84	34	94	176	185	61
		130-139	20	6	148	206	158	98
		140-149	14	2	175	216	151	117
		150-159	2		167	191	109	134
		160-169	1		185	172	72	185
		170-179			157	114	54	184
		180-189			150	63	42	178
		190-199			96	27	36	143
		200-209			83	18	22	125
		210-219			40	7	20	79
		220-229			27	3	6	53
		230-239			8		2	26
		240-249			5		2	8
		250-259			0		2	9
		260-269			0			5
		270-279			1			
		280-289			1			
		290-299			2			
		300-309			0			
		310-319			1			
Total	1,509	Total	1,515	1,516	1,517	1,519	1,517	1,516
Mean yield per tree	pounds 20.91 ±0.234		pounds 82.25 ±0.453	pounds 73.44 ±0.404	pounds 158.99 ±0.576	pounds 141.45 ±0.482	pounds 127.55 ±0.617	pounds 170.50 ±0.603

\* Crop picked in the spring of years mentioned.

each row separately, according to the condition of the soil in the various sections of the field.

The entire orchard was maintained with uniform culture until the spring of 1927. During this preliminary period great care was taken to

TABLE 2  
COEFFICIENTS OF VARIATION OF YIELDS OF INDIVIDUAL TREES

Year	Coefficient of variation, per cent
1921	64 35±1 07
1922	31 79±0 43
1923	31 78±0 43
1924	20 93±0 27
1925	19 71±0 25
1926	27 93±0 37
1927	20 41±0 26

TABLE 3  
MEAN YIELD IN POUNDS PER TREE FOR EACH PLOT, 1921

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2	18	34	27	28	15				9	25
4	22	35	19	22	40				7	24
6	29	36	35	24	40				12	19
8	14	36	34	24	31				15	15
10	23	31	20	14	16				19	12
12	30	16	25	28	12				13	17
14	12	23	32	32	13	15			17	19
16	19	30	27	21	14	19			13	20
18	15	33	27	32	20	23			13	6
20	16	22	24	28	15	21			9	18
22	15	31	19	28	28	20	10		19	11
24	25	*	21	34	22	17	18		24	20
26	20	25	28	36	23	28	19			14
28	23	24	24	37	20	29	18			18
30	32	28	32	36	37	38	24			21
32	30	33	29	23	21	26	18	23		18
34	22	*	29	34	12	27	25	19		15
36	20	27	31	15	27	25	20	14		12
38	18	22	17	24	16	23	20	15		17
40	20	22	24	20	26	37	22	13		7
42	21	25	27	28	26	25	32	16		5
44	14	17	21	31	24	†	21	23		5
46						15	16	11		6
48						15	11	7		4
50						13	10	8		5
52						16	12	4		7
54						5	‡	*		7

\* Plots omitted because of injury to trees.

† Omitted from present calculations because of injury.

‡ Fruit stolen.

give all trees the same attention. The various orchard practices were carried out with moderation. Pruning was very light, only enough being done to build trees of good structure and to remove dead wood. Considerable attention was paid to the elimination of accidental factors which might affect yield. Careful examinations of all trees were made periodically for accidental defects and disease. In addition, study was

TABLE 4  
MEAN YIELD IN POUNDS PER TREE FOR EACH PLOT, 1922

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2.....	66	88	75	89	90				41	71
4.....	76	86	79	82	112				36	64
6.....	87	97	81	82	123				47	64
8.....	51	93	80	93	104				66	68
10.....	69	92	91	83	91				63	68
12.....	86	75	100	120	85				60	63
14.....	55	82	111	108	75	100			65	73
16.....	66	95	106	89	83	104			63	77
18.....	59	96	97	102	89	105			59	49
20.....	59	73	95	106	85	98			44	66
22.....	60	95	93	98	105	76	75		57	55
24.....	68	*	82	103	82	89	97		74	73
26.....	58	85	78	108	93	101	77			75
28.....	72	72	78	115	88	115	103			58
30.....	84	88	97	105	114	125	95			82
32.....	78	86	102	84	78	107	80	111		77
34.....	62	*	87	108	68	104	100	93		72
36.....	77	93	91	81	117	101	84	93		67
38.....	70	72	61	70	87	99	96	88		88
40.....	75	76	80	68	90	110	94	82		58
42.....	68	80	92	92	93	94	105	98		57
44.....	62	79	78	64	83	†	93	104		58
46.....						86	97	91		46
48.....						88	84	91		51
50.....						83	79	82		61
52.....						83	89	78		62
54.....						65	66	*		47

\* Plots omitted because of injury to trees.

† Omitted from present calculations because of injury.

undertaken to analyze the causes of the differences in yields which were recorded. This consisted of systematic soil surveys, studies on soil moisture, determination of soil nitrates, and inspection for differences in relative infestation of the citrus nematode *Tylenchulus semipene-trans*, in high and low-yielding plots. None of these factors was considered to be the primary cause of the variations in yield.

During the period until the Washington Navel orange crop was harvested in the spring of 1927, the responses of the test trees were

measured by three criteria. These were: (1) the volume of the top of the tree expressed in cubic feet (determined by a canvas drawn over the top of the tree); (2) the area of the cross section of the trunk of the tree at a marked point; and (3) the yield of the trees. The yields during the first two years, 1921 and 1922, were taken carefully on a volume basis. One-tenth of a picking box was used as a unit. This value was later mul-

TABLE 5  
MEAN YIELD IN POUNDS PER TREE FOR EACH PLOT, 1923

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2	50	76	60	72	63				61	84
4	66	58	72	63	62				84	100
6	74	65	52	56	79				78	89
8	52	60	42	66	68				86	71
10	65	73	65	73	55				82	78
12	71	59	72	79	55				103	87
14	46	75	85	86	51	54			96	103
16	57	51	83	74	75	56			73	78
18	47	61	64	75	60	58			77	62
20	62	62	72	81	68	65			65	51
22	66	107	80	91	86	65	65		64	65
24	82	*	58	56	86	59	60		75	62
26	69	87	56	62	64	63	46			62
28	75	83	95	93	75	78	88			58
30	55	92	114	72	103	83	92			79
32	74	84	95	83	81	76	88	72		85
34	56	*	89	78	69	73	75	48		75
36	77	85	90	93	104	83	73	54		86
38	77	78	68	69	88	82	90	59		80
40	80	69	85	87	82	89	72	56		83
42	82	65	86	84	86	84	86	70		76
44	70	69	73	72	58	†	73	102		79
46						49	75	72		89
48						59	78	74		67
50						67	81	63		61
52						46	90	79		75
54						67	79	*		59

\* Plots omitted because of injury to trees.

† Omitted from present calculations because of injury.

tiplied by the average value for the weight of this volume of fruit (4.244 pounds) so that the yield might be expressed in pounds. Beginning with 1923, the total amount of fruit produced by each tree, including windfalls, was weighed.

The yields for the period of seven years, 1921 to 1927 inclusive, were obtained prior to the time of applying the various fertilizer treatments in 1927. Considering the field as a uniformity, or blank, experiment, the data have been subjected to a study of some factors which might influence the accuracy of the future trials.

The records of all normal trees of the same age are recorded for the purpose of this study. Certain trees, during the course of ten years, have naturally suffered from accidental causes, particularly from gopher injury, trunk and root diseases, and cultivation accidents. Some of these trees have been replaced by young ones, the records of which are omitted here. Others have recovered to a normal condition and their

TABLE 6  
MEAN YIELD IN POUNDS PER TREE FOR EACH PLOT, 1924

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2	105	133	120	136	149				140	165
4	109	114	145	126	145				198	184
6	142	132	135	125	162				179	209
8	104	130	118	149	171				189	179
10	119	138	142	135	146				179	180
12	114	127	140	161	151				189	191
14	113	146	169	158	143	175			177	203
16	127	154	165	161	153	175			120	138
18	110	126	138	153	156	173			159	142
20	122	146	159	171	165	181			151	141
22	124	179	163	179	183	156	171		161	159
24	138	*	153	141	158	153	167		152	141
26	122	145	120	127	147	147	131			157
28	143	154	164	166	172	186	199			155
30	129	166	190	162	196	203	182			174
32	149	155	167	168	162	160	186	189		188
34	142	*	172	158	142	181	174	150		157
36	153	172	187	193	186	185	174	162		171
38	143	167	146	159	172	179	192	159		148
40	134	158	158	176	175	191	177	157		160
42	128	140	189	178	177	178	193	180		158
44	117	137	172	171	153	†	191	198		181
46						159	184	184		188
48						150	190	177		149
50						168	172	163		136
52						147	191	193		160
54						157	182	*		140

\* Plots omitted because of injury to trees.

† Omitted from present calculations because of injury.

records, which were temporarily excluded from the calculations, are included in the later years. All obvious cases of bud-mutation have been eliminated. The elimination of the records of 7 abnormal trees, only, has been necessitated by factors of an unknown nature. The effect of deletion of the yield of abnormal trees upon total plot yield has been compensated for by considering the plot yields on the basis of mean yields per tree. This procedure gives equal weight to the records of individual plots when they are combined.



When there were more than 4 abnormal trees in any one plot, the entire plot was eliminated from the records for the purposes of the present study. Four plots were eliminated for this reason during the entire period, and in addition, 1 plot was eliminated in the year 1921 because of the theft of the matured fruit. Two plots contain only 4

TABLE 7  
MEAN YIELD IN POUNDS PER TREE FOR EACH PLOT, 1925

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2	121	138	125	128	145				109	115
4	116	135	136	125	151				152	150
6	129	132	136	114	171				120	138
8	95	128	121	140	164				156	148
10	115	120	128	124	145				138	138
12	113	134	129	145	141				160	148
14	107	158	149	149	131	168			129	136
16	119	143	143	134	123	161			105	101
18	105	133	130	122	135	145			138	102
20	106	132	136	155	135	151			124	103
22	109	141	148	144	153	127	140		142	139
24	115	*	133	127	116	139	145		135	115
26	101	116	106	108	116	120	116			151
28	131	128	143	136	149	158	161			126
30	121	147	159	140	155	181	159			152
32	138	146	151	158	140	142	151	201		150
34	129	*	153	150	134	170	155	155		125
36	157	163	174	175	158	168	149	175		146
38	133	153	138	156	152	165	170	159		144
40	137	153	156	166	171	174	158	160		116
42	127	143	187	170	157	160	164	165		158
44	126	142	157	166	140	†	152	179		164
46						138	166	171		141
48						140	168	163		129
50						153	147	153		117
52						137	175	170		124
54						123	149	*		115

\* Plots omitted because of injury to trees.

† Omitted from present calculations because of injury.

normal trees, 1 contains 5, from 5 to 7 plots contain 6 in various years, and from 18 to 22 contain 7, while from 164 to 167 plots contain the full number, 8 trees.

## STUDIES OF VARIABILITY OF YIELDS

Munson <sup>(36, 37)</sup> was among the earliest investigators to call attention to the marked difference in the yield of trees given the same cultural care. More recently the extent of the normal variation existing in uniformly treated orchards has been studied statistically by several

authors. Among these are Pickering,<sup>(39)</sup> Batchelor and Reed,<sup>(4)</sup> Sax and Gowen,<sup>(48)</sup> Grantham and Knapp,<sup>(14)</sup> Anthony and Waring,<sup>(3)</sup> and Gadd.<sup>(13)</sup> Although the plantings studied have generally been selected for experimental purposes, and many of them have probably been more uniform than the average of commercial orchards, the results obtained

TABLE 8  
MEAN YIELD IN POUNDS PER TREE FOR EACH PLOT, 1926

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2.....	72	81	86	90	103				113	133
4.....	75	93	111	98	106				128	152
6.....	96	78	95	103	119				115	141
8.....	62	109	84	110	107				143	144
10.....	77	92	97	109	126				134	134
12.....	93	93	114	131	113				122	126
14.....	79	107	121	122	101	123			125	137
16.....	75	85	107	110	106	101			97	101
18.....	75	95	99	110	116	126			131	115
20.....	85	105	115	131	127	112			121	109
22.....	94	124	122	122	136	112	110		120	124
24.....	103	*	112	103	93	87	109		117	128
26.....	97	118	96	93	95	89	77			150
28.....	108	121	115	107	117	110	126			133
30.....	94	129	141	124	151	133	143			143
32.....	126	135	130	128	117	115	133	156		154
34.....	146	*	151	128	128	129	140	130		148
36.....	160	164	142	155	132	128	130	148		152
38.....	129	166	128	135	141	141	155	135		146
40.....	139	156	149	156	144	150	126	141		147
42.....	122	142	185	154	146	152	179	192		182
44.....	125	139	171	153	139	†	170	199		165
46.....						148	199	212		195
48.....						163	180	161		153
50.....						167	189	169		142
52.....						136	177	185		145
54.....						152	180	*		133

\* Plots omitted because of injury to trees.

† Omitted from present calculations because of injury.

have caused these authors to emphasize the magnitude of chance variations. The results also show that the extent of fortuitous variations is itself very different in the various orchards. Thus the coefficient of variation of individual trees has been reported to lie within the extremely broad range from 19.66 per cent (73 Jonathan apple trees for thirteen years, data of Anthony and Waring<sup>(3)</sup>) to 89.6 per cent (882 Ben Davis apple trees for 1918 only, reported by Sax and Gowen<sup>(48)</sup>). The majority of the coefficients given by Batchelor and Reed,<sup>(4)</sup> and by Anthony and Waring,<sup>(3)</sup> lie between 30 and 50 per cent. The limited

data of this nature which are available suggest that the extent of variation fluctuates within different limits for each planting in various seasons. A knowledge of the characteristics of each orchard would apparently, therefore, be an aid in the planning of experimental work and the interpretation of the results obtained.

TABLE 9  
MEAN YIELD IN POUNDS PER TREE FOR EACH PLOT, 1927

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2	108	104	107	103	128				138	159
4	128	152	169	129	144				165	188
6	136	153	156	114	183				156	204
8	107	153	146	129	157				192	210
10	145	162	174	135	168				184	169
12	134	168	179	134	132				206	184
14	159	186	186	145	146	170			203	173
16	154	167	170	144	140	182			139	145
18	159	180	145	152	164	186			179	160
20	152	177	187	164	167	181			189	157
22	135	180	185	186	163	162	161		187	199
24	114	*	186	185	154	158	159		157	209
26	135	173	168	160	186	171	172			183
28	183	157	181	177	172	180	166			165
30	167	164	186	174	193	194	168			188
32	195	158	165	185	178	172	150	180		178
34	177	*	190	182	187	176	163	191		188
36	188	202	193	186	203	193	152	193		191
38	170	186	172	155	177	187	185	171		181
40	160	217	182	180	187	188	168	187		168
42	154	198	205	195	204	183	205	214		198
44	141	189	206	181	181	†	185	210		219
46						178	209	201		216
48						174	197	173		162
50						163	184	156		172
52						136	179	187		168
54						118	160	*		152

\* Plots omitted because of injury to trees.

† Omitted from present calculations because of injury.

#### VARIABILITY OF TREE YIELDS

The frequency distributions of yields of single trees of the planting under consideration are given in table 1 for the years 1921 to 1927 inclusive. During 1921 the trees produced the initial crop. Many trees produced less than 10 pounds per tree, and a considerable proportion of the trees produced nothing. (See table 3.) Inspection of table 1 indicates that in each year, except 1921, distributions were obtained which approach the distribution of the normal curve. In the years

1922 to 1927 inclusive, the application of the methods of statistics, based upon the assumption of a normal distribution to the problems undertaken, is apparently valid.

The coefficients of variation for annual yields of single trees in the seven years are given in table 2. In calculating them, the usual formula<sup>5</sup> for the coefficient was used, regardless of the type of the frequency distribution.

The first striking fact noted is, perhaps, the extremely large amount of variation of yields during the first year, 1921. In subsequent years the coefficients are less than half that of the first year. A tendency for the coefficients to be smaller after the third crop has been harvested is also shown. With the exception of 1926, the coefficients are about equal for the last four years of the period. The year 1926 was one of rather small crops, and it is probable that the influences reducing the size of crop that year may have been effective in increasing the variation.

If the coefficients for the year 1921 are excluded, the mean of the constants for the remaining six years is 25.4 per cent. In most of the orchards for which data on variability are available, the trees have been adjacent to each other. Although planting distances have varied in such trials, the trees have usually been larger and had a more extensive root system, so that the actual areas between test trees may have been comparatively small. In the present case, however, the field covers a relatively large area, and the test trees are only 40 per cent of the entire number. It is logical to assume that the use of an increased area of land (necessitated by the use of guard rows) would ordinarily cause an increase in variation of the test trees, by virtue of this greater dispersion. The relatively low coefficients obtained, therefore, are considered as evidence indicating the effectiveness of the original plan and the management of the planting in obtaining an uncommonly uniform orchard.

#### VARIABILITY OF PLOT YIELDS

The mean yields per normal tree of each plot for the seven years, 1921 to 1927 inclusive, are given in tables 3 to 9. The frequency distributions of these mean yields are presented in table 10.

The type of distribution obtained for yields of the year 1921 on a plot basis (table 10) is markedly in contrast with that obtained on a

$$C = \frac{\sqrt{\frac{\sum fd^2}{N}}}{M} \cdot 100; \text{ while } E_c = \pm \frac{0.6745}{\sqrt{2N}} C \left[ 1 + 2 \left( \frac{C}{100} \right)^2 \right]^{\frac{1}{2}}$$

tree basis for the same year (table 1). It is evident that trees of zero or very low productivity were not as a rule grouped together in local areas.

For the years 1922 to 1927 inclusive, the distributions on a plot basis approach the distribution of the normal curve. The use of the usual

TABLE 10  
FREQUENCY DISTRIBUTION OF YIELDS PER TREE PER PLOT FOR EACH YEAR,  
1921 to 1927

Mean yield per tree per plot, pounds	Number of plots, 1921	Mean yield per tree per plot, pounds	Year					
			1922	1923	1924	1925	1926	1927
			Number of plots					
0-4	2	30-39	1					
5-9	14	40-49	6	7				
10-14	24	50-59	13	28				
15-19	43	60-69	28	44			1	
20-24	48	70-79	33	53			8	
25-29	31	80-89	44	44			7	
30-34	20	90-99	36	11		1	19	
35-39	10	100-109	23	7	3	12	19	5
40-44	2	110-119	8	1	7	15	23	3
		120-129	3		14	29	32	4
		130-139			14	32	23	10
		140-149			30	35	23	10
		150-159			33	37	17	25
		160-169			24	18	8	30
		170-179			31	13	4	27
		180-189			22	2	6	48
		190-199			14	0	4	15
		200-209			3	1	0	13
		210-219					1	5
Total	194	Total	195	195	195	195	195	195
Mean yield per tree per plot	pounds 21.2±0.39	Mean yield per tree per plot	pounds 82.5±0.87	pounds 73.0±0.67	pounds 158.6±1.10	pounds 141.3±0.97	pounds 127.2±1.39	pounds 170.1±1.15

statistical methods is apparently justifiable with this material in these years. The distributions are not so smooth as the ones for the yields of individual trees in the same years (table 1), however, a condition which is probably due to the smaller number of individuals in the populations.

The extent of variation as measured by the coefficient of variation has been determined for the mean yield per tree for each plot, during the respective years. The constants obtained are presented in table 11. It may be noted that the coefficients show the same trend as those obtained

for yields of single trees in the corresponding years (table 2). They are, however, all lower on a plot-mean basis than on the basis of individual trees.

If the yields of the trees were such that they were distributed at random throughout the orchard, the coefficient of variation of the plots ( $C_p$ ) should tend to approximate the values given by the formula

$$C_p = \frac{C_t}{\sqrt{n}},$$

where  $n$  is the number of trees per plot and  $C_t$  the coefficient of variation of tree yields. The theoretical coefficients of variation calculated by this formula (table 11) may be compared with the values actually found. One may observe that in each year the actual variation is greater than the theoretical. From this it is evident that a degree of correlation exists between the yields of trees of the same plots, because of the influence of some factor which tends to equalize their yields.

It is possible to determine the extent of the correlation between yields of trees of the same plot by means of the formulas for intraclass correlation developed by Harris<sup>(16)</sup> for use as a criterion of the homogeneity of fields. Application of his formula<sup>6</sup> resulted in the constants recorded in table 12.

A very significant positive correlation is seen to exist between the yields of trees of the same plots in each year. The mean correlation for

<sup>6</sup> On account of the varying number of trees in the plots the formula used was:

$$r_{p1p2} = \frac{\{ [\Sigma(C_p^2) - \Sigma(p^2)] \div \Sigma[n(n-1)] \} - \bar{p}^2}{\sigma_p^2}$$

where

$$\bar{p} = \Sigma[(n-1)p] \div \Sigma[n(n-1)]$$

$$\sigma_p^2 = \frac{\Sigma[(n-1)p^2]}{\Sigma[n(n-1)]} - \left( \frac{\Sigma[(n-1)p]}{\Sigma[n(n-1)]} \right)^2$$

in which

$r_{p1p2}$  = mean correlation between the yields of trees of the same plot

$C_p$  = total yield of plots

$p$  = yield of individual trees

$n$  = number of  $p$  in the proper plot

$\bar{p}$  = mean of yields of trees

The constants were calculated from actual values. It was originally thought that the process should be materially simplified and that they could be calculated from grouped data in which trees in plots of either 8, 7, 6, 5, or 4 trees each, were distributed separately, and plot yields were also so distributed. It was found, however, that grouping reduced values for  $\sigma_p^2$  markedly, and values obtained for  $r_{p1p2}$  were too high.

The coefficients for 1921 and 1922 were determined from records of yield based on a volumetric unit, since it was thought that the use of the factor in converting volume records to weight might have introduced a certain degree of correlation into the distributions. This appeared to be the case; for when the calculated weights were used, the values of  $r_{p1p2}$  found were slightly, if not significantly, higher, being  $+0.353 \pm 0.015$  for 1921, and  $+0.298 \pm 0.016$  for 1922.

the seven years is  $+0.332$ . The high correlation of  $+0.537 \pm 0.012$  which existed in 1926, a year in which yields were below normal, suggests that the crop that year in the experimental orchard was influenced to an unusual extent by circumstances which affected some regions in the field more than it did others. Variation was also greater in 1926 than in any other year from 1923 to 1927.

TABLE 11  
COEFFICIENTS OF VARIATION OF YIELDS OF PLOTS

Year	Observed coefficient of varia- tion, per cent	Theoretical ( $C_{tree} \div \sqrt{8}$ ) per cent
1921.....	37.51 $\pm$ 1.45	22.75
1922.....	21.68 $\pm$ 0.77	11.24
1923.....	19.03 $\pm$ 0.67	11.24
1924.....	14.36 $\pm$ 0.50	7.40
1925.....	14.15 $\pm$ 0.49	6.97
1926.....	22.58 $\pm$ 0.81	9.88
1927.....	13.95 $\pm$ 0.49	7.22

TABLE 12  
COEFFICIENTS FOR INTRAClass CORRELATION\* OF YIELD OF TREES WITHIN THEIR  
RESPECTIVE PLOTS

Year	Coefficient of correlation
1921.....	+0.316 $\pm$ 0.016
1922.....	+0.269 $\pm$ 0.016
1923.....	+0.132 $\pm$ 0.017
1924.....	+0.340 $\pm$ 0.015
1925.....	+0.370 $\pm$ 0.015
1926.....	+0.537 $\pm$ 0.012
1927.....	+0.359 $\pm$ 0.015
Mean.....	+0.332

\* Calculated according to Harris' formulas.

When the average coefficient of correlation ( $r$ ) between the trees of the plots is known, it is possible to calculate the expected coefficient of variation of the plots ( $C_p$ ) on the basis of the observed variation of the individual trees ( $C_p$ ) as given in table 2. This has been done by means of the formula (after Yule<sup>(70)</sup> p. 286),

$$C_{C_p}^2 = \frac{C_p^2}{n} [1 + (n-1)r],$$

using the coefficients noted above in table 12. The calculated values for the coefficient of variation are given in table 13. They differ slightly from the observed constants given in table 11, but are of the same order.

The correlations which exist between the yields of trees in the same plot emphasize the magnitude of systematic variation in the annual yields of the trees of this planting. As an effort was made to plant the trees at random, there appears to be no principal factor other than soil differences which would cause this type of correlated variation. The importance of soil variability is, therefore, stressed. In the Harris coefficients of intraclass correlation, there is a suggestion that this influence

TABLE 13

CALCULATED COEFFICIENTS OF VARIATION OF PLOTS BASED UPON THE VARIABILITY OF INDIVIDUAL TREES AND THE INTRACLASSE CORRELATION

Year	Coefficient of variation, per cent
1921 .....	40.60
1922 .....	19.08
1923 .....	15.59
1924 .....	13.60
1925 .....	13.20
1926 .....	21.54
1927 .....	13.52

may be more apparent in some years than in others. It is reasonable to assume from this information that if some areas maintain more vigorous trees than others, they will not suffer to the same extent in years of climatic stress. Soil differences, therefore, may be relatively more effective in causing systematic variation under adverse conditions.

#### VARIATION IN DIFFERENT YEARS

The practical value of the uniformity experiment lies partly in the fact that it may disclose areas of land which are not fitted for experimental purposes. In the areas which are suitable, it may also give some knowledge of the variability existing during the period of observation. The application of the latter information to the future trials rests upon the assumption that the extent of the variation and hence, presumably, the nature of the variations, will tend to be approximately the same in different years or periods.



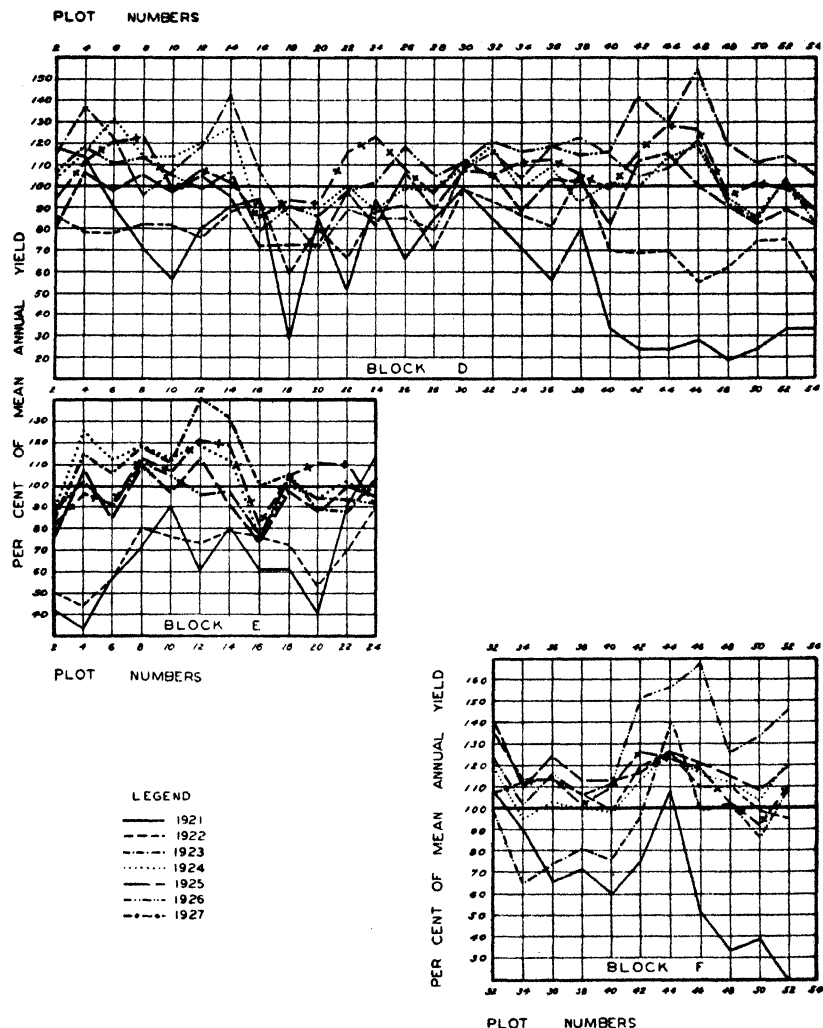


Fig. 4. Mean annual yield per tree for each plot in Blocks D, E, and F in percentage of the respective annual mean of the 195 plots of the orchard, for the years 1921 to 1927.

The data given for the variability of trees and plots of the orchard under discussion indicate, if the records of 1921 are not considered, that although there is some fluctuation in the coefficients of variation in individual years, there is a tendency for the annual gross variation to be of somewhat the same order. Similar conclusions were reached by Batchelor and Reed<sup>(4)</sup> and are made apparent by a study of the data of Sax and Gowen<sup>(48)</sup> on the variability of several orchards. The question

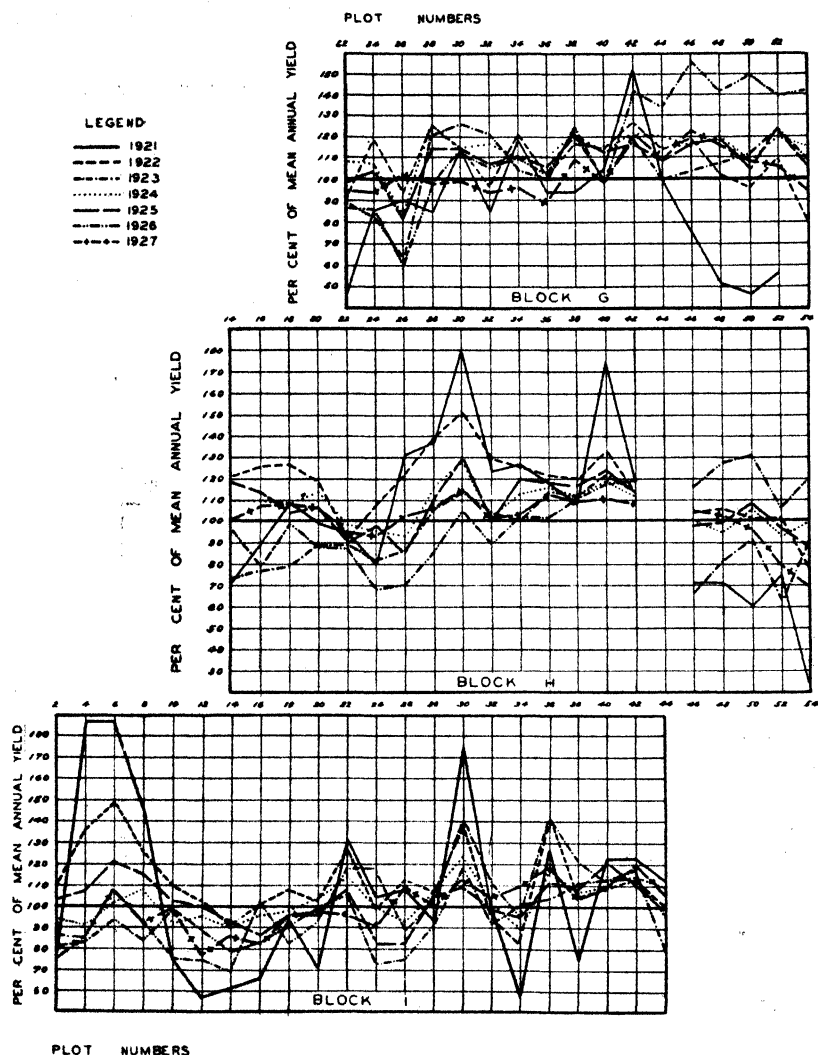


Fig. 5. Mean annual yield per tree for each plot in Blocks G, H, and I in percentage of the respective annual mean of the 195 plots of the orchard, for the years 1921 to 1927.

naturally arises as to whether this tendency towards somewhat constant gross variation is due to more or less consistent differences in relative yield of various plots and trees, or whether the individual fluctuations in yield are due to mere chance.

A comparison of the annual production of fruit of individual plots of all blocks of the orchard under discussion is given in figures 4, 5, and 6. In order to place the data on a comparable basis, the mean

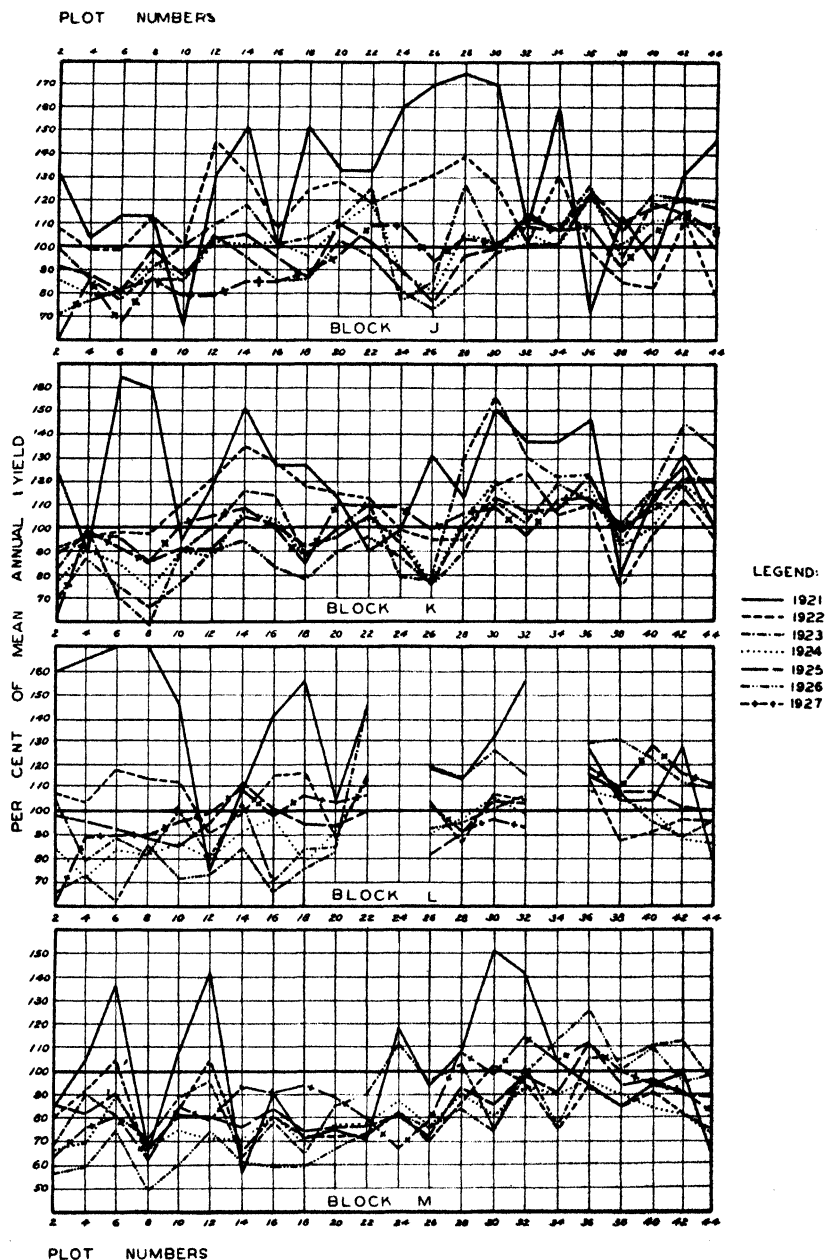


Fig. 6. Mean annual yield per tree for each plot in Blocks J, K, L, and M in percentage of the respective annual mean of the 195 plots of the orchard, for the years 1921 to 1927.

yields per tree of each plot are expressed in percentage of the mean yield per tree of all plots for the entire field for the proper year. With the exception of the yields of 1921, a tendency for individual plots to yield within a somewhat limited range is observed. This is particularly marked for the yields of 1924 to 1927 inclusive. The relative yields of the plots in 1922 and 1923 depart considerably from the values for later years, but these departures are of much less magnitude than those observed for the year 1921.

The tendency of the plots of this comparatively uniform orchard to yield about the same relative amount of fruit in the years 1922 to 1927 inclusive, can be emphasized by the calculation of the errors of the relative yields. Using the figures of the mean annual yield per tree per plot expressed in percentage of the mean plot yield of each year, which are plotted in figures 4, 5, and 6, the average probable error of the yield of a single plot in one year is given by the formula :

$$E_s = \pm 0.6745 \frac{\Sigma \left( \frac{\sum d^2}{n} - c^2 \right)^{1/2}}{N},$$

where

$N$

$E_s$  = average probable error of the yield of a single plot about the mean yield of that plot in percentage of the mean annual plot yield

$d$  = deviation of the yield of each plot in each year from the guessed mean percentage yield of that plot

$c$  = correction to the guessed mean yield of the plot

$N$  = number of plots (195)

$n$  = number of years (6)

Calculation by the above formula, using the data indicated, shows that the average probable error of the yield of a single plot in any one year, around its own mean yield ( $E_s$ ), equals 8.46 per cent of the mean yield of all plots. If the yields of all six years are combined to obtain the average probable error of the mean yield per tree for each plot for the six-year period, 1922 to 1927 inclusive, which may be called  $E_m$ , then,

assuming a normal distribution,  $E_m = \frac{8.06}{\sqrt{6}} = 3.29$  per cent of the mean

plot yield for this period. With this information available, it is evident by inspection of figures 4, 5, and 6 that there is a tendency for the plots to yield about the same relative amounts, and that there were significant differences in mean yield between many of the plots during the preliminary period of testing.

For measuring the interannual relations between the responses of plants, Harris<sup>(15)</sup> has urged the use of the coefficient of correlation. Harris and Scofield<sup>(18, 19)</sup> have applied the method to the study of the permanence of yields of field crops. They find that in general there is a positive correlation between the yields of plots throughout a term of years, but that the correlation is influenced by weather conditions and by the nature of the rotation of the crops. Following the same procedure, Sax and Gowen<sup>(48)</sup> found that with apple trees on their experimental farm a high correlation exists between yields of the same trees over a period of five years. They reported similar findings as a result of studies of data of Hedrick and Anthony<sup>(21)</sup> for apples, and of data of Shamel, Scott, and Pomeroy<sup>(49, 50)</sup> for Washington Navel and for Valencia oranges. Collison and Harlan<sup>(8)</sup> have recently published simi-

TABLE 14

INTERANNUAL CORRELATION COEFFICIENTS FOR YIELDS OF INDIVIDUAL TREES

	1922	1923	1924	1925	1926	1927
1921	+0 637±0 010	+0 260±0 016	-0 173±0 017	+0 170±0 017	-0 171±0 017	-0 083±0 017
1922		+0 307±0 016	+0 324±0 016	+0 455±0 014	+0 069±0 017	+0 153±0 017
1923			+0 595±0 011	+0 415±0 014	+0 347±0 015	+0 255±0 016
1924				+0 685±0 009	+0 532±0 012	+0 466±0 014
1925					+0 550±0 017	+0 488±0 013
1926						+0 536±0 012

larly high interannual correlations in yield of trees of the experiment station at Geneva, New York.

In order to determine the relation of the yield of one year to that of the other years for the present orchard, the possible interannual correlations of yield of individual trees have been calculated. These are presented in table 14.

With the exception of the yields of 1921 as compared with those of the other years, significantly positive correlations were found. Correlations involving yields of 1921 showed considerable irregularity, ranging from a very significant positive coefficient with yields of trees in 1922 to significant negative correlations in three of the subsequent years. No consistent relation of the yields of 1921 with those of later years is indicated by the data.

Correlations obtained for the yields of trees in 1922 with the yields in following years are all significantly positive, although one correlation (with the yields harvested in 1926) is relatively low, being only slightly larger than four times its probable error. All the possible correlations

found for yields in 1923 with those of subsequent years are positive, high, and significant. There is a suggestion that the magnitude of the correlations decreases with time, however, for the correlations between yields of consecutive years were found to be largest, while the correlations decrease slowly as comparisons are drawn with more remote years. There are no irregularities of such magnitude as those noted for correlations involving yields of the first year. The next most erratic behavior is for correlations involving yields of the year 1922.

The calculation of interannual correlations for the yields per tree of plots makes similar facts manifest. The coefficients obtained on a plot basis are of the same order as those obtained on an individual tree basis. As indicated in table 15, the values obtained for correlations of

TABLE 15

INTERANNUAL CORRELATION COEFFICIENTS FOR MEAN YIELD PER TREE OF PLOTS

	1922	1923	1924	1925	1926	1927
1921	+0.644±0.028	+0.168±0.047	-0.065±0.048	+0.114±0.048	-0.272±0.045	-0.002±0.048
1922		+0.173±0.047	+0.272±0.045	+0.486±0.037	+0.033±0.048	+0.014±0.048
1923			+0.597±0.031	+0.385±0.041	+0.402±0.041	+0.331±0.043
1924				+0.740±0.022	+0.688±0.026	+0.605±0.031
1925					+0.639±0.029	+0.554±0.034
1926						+0.606±0.031

the yield of plots in 1921 with the yield of similar plots in subsequent years are irregular. Similar correlations of plot yields in the year 1922 with those of following years are also variable, those for three years being positive, although small with the exception of the correlation with 1925, while with 1926 and 1927 the values are practically zero.

The correlations between plots involving all possible combinations of 1923 and later years are all positive and highly significant. In general, the values are highest for consecutive years and decrease slowly as the interval between the years increases. The ultimate limits of this tendency are, of course, a matter of conjecture. The actual magnitude of the correlations and their slow rate of decrease strongly suggest that a positive correlation may exist for a considerable period.

The agreement between the interannual correlations of yield of trees on one hand and those of plots or "plot averages" on the other, for the same periods, is additional evidence indicating that soil variability is the most important factor determining relative yields of plots. This appears to be reasonable in view of the high correlation existing between trees of the same plot in each year.

THE VALUE OF THE RECORDS OF YIELD IN VARIOUS INDIVIDUAL YEARS IN THE  
ANALYSIS OF THE PRODUCTION OF THE ORCHARD

It has been observed that the tendency for the relative yields of the trees and plots to approach the same value for the greater part of the period prior to the beginning of the experiment is not substantiated by the production of the trees in the year 1921. It seems that factors are operative which do not affect the subsequent yields. The frequency distribution of yields of individual trees also suggests that other factors, such as those concerned with the physiological condition of fruiting or nonfruiting, are operative. Many trees did not bear in 1921, and many more bore only a very small quantity of fruit.

The irregular relations of the yields of that year with the yields of other years suggest that production during the first year of bearing is not a reliable index of the performance of the trees in later periods. Had the field been under differential treatments, very different conclusions might easily have been inferred from the 1921 results than from those of following years. It seems, therefore, that the yields of these trees during the first year of production do not provide a reliable basis for the prediction of the responses of more mature trees. These records are not used, therefore, in calculations upon the reliability of various ways of laying out the field, treated in a later section of this paper.

Some question may possibly be raised, also, as to the reliability of the use of the yields of the second and the third years as indexes of productiveness of the trees. A considerable number of trees did not come into production until 1922. An additional factor which may have been of considerable importance in this respect was the effect of a freeze which occurred in January, 1922. The greatest amount of the damage occurred in the blocks west of the canal, where the fruit picked in 1922 was rendered unfit for sale. However, the weights of all the fruit were recorded soon after the freeze. Some damage to twigs was experienced, and this was also more severe west of the canal. Since the installation of orchard heaters in 1923, no further damage from this source has occurred.

Variability was greater in 1922 and 1923 than in following years. However, the graphs of the yields of those years (figs. 4, 5, and 6) indicate in most cases a tendency towards parallelism with the graphs of yields of later years, although there are striking exceptions, particularly for yields of 1922. The correlations between yields of trees in 1922 and yields in later years were positive and significant, though they were

low in the more remote years. In two years the correlations between yields per tree of plots in 1922 and those in later years failed to be significant. The yields of trees and plots in 1923 were positively correlated with the yields in subsequent years. The curves which might be made from the yields of the trees in 1922 and 1923 would be nearly normal in type.

The factors which might cause exceptional responses in 1922 and 1923 may be of considerable importance in determining the reliability of the yields of these years. However, it has not been possible to separate the effect of "usual" seasonal influences on yield, which it is desirable to sample, from the effects of the physiological conditions accompanying the attainment of the fruiting condition and of the freeze in 1922. The influence of the changes accompanying fruiting are, however, not nearly so important in the later years as in 1921, judging by the frequency distributions. The nature of the interannual correlations suggests that the conditions affecting the responses of the trees in later years were also operative to an important extent during 1922 and 1923. Perhaps, therefore, it is safer to use the records for these two years, combined with the yields of the succeeding four years, as an index of productivity. No serious consequence would seem to result from this decision, although total variation may be slightly increased, and there is a possibility that correlations during the period for which the index is taken and the subsequent years may be reduced thereby.

There appears to be no objection to the use of yield records of the fourth to the seventh years as indicating, in part, the productivity of the trees during this preliminary period. The parallelism of the yield of groups of trees, the high interannual correlations existing, the nearly normal distributions noted, and the similarity of the coefficients of variation in the individual years all seem to indicate that in these years the responses of the trees and plots might well be obtained as samples of the same population. All are influenced chiefly by factors of a climatic nature and factors concerning the normal growth of the trees. These cause only limited fluctuations in the yield of individuals in various years.

#### THE VALUE OF THE AVERAGE YIELD OF SEVERAL YEARS

There is always involved in a group of field experiments the question of the accuracy of the trial during the period of its duration, and also the question of the probable results of a similar experiment over another series of years in the same location. Stadler<sup>(11)</sup> has emphasized the importance of the seasonal fluctuations in the responses of cereal crops,



while Engledow and Yule<sup>(11)</sup> have clearly pointed out that the reliability of a prediction based upon the results of an experiment cannot be less than the error entailed by the sample of the seasons involved during the period of the experiment. In the case of orchard crops it is fre-

TABLE 16  
MEAN ANNUAL YIELD IN POUNDS PER TREE OF EACH PLOT\* FROM 1922 TO 1927

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2	87	103	96	103	113				100	121
4	C-95	106	119	104	C-120				C-127	140
6	111	110	C-109	99	140				116	141
8	79	112	99	C-115	129				139	C-137
10	98	C-113	116	110	122				130	128
12	102	109	122	128	113				140	133
14	93	126	137	128	108	132			133	138
16	100	116	129	119	113	C-130			100	107
18	93	115	112	119	120	132			124	105
20	98	116	C-127	135	125	131			C-116	105
22	98	138	132	137	138	116	120		122	124
24	C-103	†	121	119	115	114	C-123		118	C-121
26	97	121	104	110	117	115	103			130
28	119	119	129	132	C-129	138	141			116
30	108	C-131	148	130	152	153	140			136
32	127	127	135	134	126	129	133	152		139
34	119	†	140	C-134	121	139	135	128		128
36	135	147	146	147	150	C-143	127	C-138		136
38	120	137	119	124	136	142	148	129		C-133
40	C-121	138	135	139	142	150	133	131		122
42	114	128	157	146	144	142	155	153		138
44	107	126	C-143	135	126	†	C-144	165		144
46						126	155	155		146
48						C-129	140	140		C-117
50						134	142	131		115
52						114	150	C-149		122
54						114	136	†		108

Mean of entire field = 125.53

Mean of C plots = 125.88

\* Locations of check, or continuity, plots are shown by the letter C. Plots related to each continuity plot by yield during the preliminary period are enclosed by horizontal lines.

† Plots omitted because of injury to trees.

‡ Omitted from present calculations because of injury.

quently very difficult, if not impossible, to maintain an experiment during a sufficient number of years to give a reliable sample of the seasons. Such a long period as would be necessary would involve other factors of possibly great importance owing to changes in age and size of the trees and to progressive or irreversible changes in the soil, so that it might be impossible to obtain an adequate sample of seasons for purposes of generalized prediction with one planting of trees. It is felt

that the use of a longer period for this study than the six years from 1922 to 1927 inclusive might possibly result in the introduction of complications such as those mentioned or those due to the effects of prolonged malnutrition of the trees. Thus the somewhat small size and pale color of the new leaves in 1927 suggested that the trees were beginning to suffer for lack of nutrients. Therefore, the treatments were started at that time.

In order to obtain an index of the productivity of the various plots during the period of six years, 1922-1927, the annual yields per tree for each plot have been averaged. This process, by smoothing some of the chance seasonal fluctuations, should result in a more reliable sample of the productive capacity of the individual plots during the preliminary period. The values obtained for the mean annual yield per tree for each plot are given in table 16. (The table gives certain other information which is discussed later.) The average probable error of the mean yields, calculated according to the method on page 103, is 3.29 per cent, or 4.34 pounds.

When the mean yields per tree of all plots were grouped into a frequency distribution with a class interval of 10 pounds, it was observed that the distribution resembled that of the normal curve. The critical functions  $\beta_1$  and  $\beta_2$ , which serve as criteria of the curve type, were calculated according to the formulas given by Pearson<sup>(38)</sup> and by Elderton,<sup>(10)</sup> indicating:

$$\beta_1 = 0.048 \pm 0.034$$

$$\beta_2 = 2.729 \pm 0.176$$

Since  $\beta_1$  is not significantly different from zero, and  $\beta_2$  is not significantly different from 3.0 (the values of these functions for the normal, or Gaussian curve), it is evident that the distribution of the mean yields per tree of each plot for the six-year period is of normal, or practically normal, type. Therefore, the use of statistical methods involving an assumption of normality is justifiable with this material.

The following statistical constants for the mean yields per tree for all plots for the years 1922 to 1927 inclusive, are calculated by the use of a class interval of 10 pounds:

Number of plots	195
Mean yield	125.53 $\pm$ 0.787 pounds
Standard deviation	16.3 $\pm$ 0.56 pounds
Coefficient of variation	12.98 $\pm$ 0.45 per cent
Probable error of a single plot	10.99 pounds

The use of the six-year mean yield per tree for each plot results in a slight decrease in variation beyond that observed for individual years.

The failure of the variation to be reduced further by this procedure is due to the tendency of the plots to remain somewhat constant in relative yielding capacity during the period under consideration, as indicated in the previous discussion. This condition is emphasized further by use of the formula given on page 98 for the coefficient of variation of the mean of a combination of a group of related variates. The arithmetic mean of all the interannual correlations on a plot basis from 1922 to 1927 inclusive, is  $+0.435$ ; the mean of the annual coefficients of variation on the same basis is 17.62 per cent. Substituting these values in the formula and solving, the coefficient of variation of the mean yield per tree for each plot is 12.82 per cent, which is very close to the observed value of  $12.98 \pm 0.45$  per cent.

## SOME RELATIONS OF THE VARIABILITY IN YIELD OF THE ORCHARD TO THE PLAN OF THE FUTURE EXPERIMENT

The results of studies of many blank, or uniformity, experiments suggest that knowledge of the normal variations in productivity of the field would be an advantage in planning the future experiment. In view of this, Love<sup>(31)</sup> and others have repeatedly emphasized the desirability of determining the characteristics of the variability of each field by means of the uniformity trial prior to the use of the field for comparative trials.

Batchelor and Reed<sup>(4)</sup> expressed the desirability of obtaining this information for orchard trees on which experiments are to be conducted. The importance of such study upon trees has since been emphasized by Anthony,<sup>(1)</sup> Chandler,<sup>(6, 7)</sup> and Gadd.<sup>(13)</sup> The data that have been presented strongly suggest that the plans which would give the most reliable results during this preliminary period might be expected also to give good results after the start of the differential treatments.

### THE ERROR OF A SINGLE PLOT

It was noted that the probable error of a single observation of plot yields for this experiment over the six-year period, 1922-1927, is 10.99 pounds. This is 8.76 per cent of the mean annual tree yield.

From the value of the probable error of a single plot it is possible to determine the differences between any 2 plots which are theoretically necessary for any desired degree of assurance that the difference is real. Wood<sup>(69)</sup> has published convenient tables for this purpose, which were originally calculated from Sheppard's<sup>(51)</sup> distribution of the normal probability integral (see tables in Pearson,<sup>(38)</sup> or McEwen,<sup>(34)</sup> for

extended work).<sup>7</sup> Wood's tables, which are presented again for convenient reference in Appendix A, give the ratio of the difference between any two means to the probable error of that difference for several levels of significance. The direction of the differences is determined in agricultural experiments by observation. In order to determine the odds that the difference is real the values in the lower half of Wood's table would be used.<sup>8</sup>

Since the probable error of a difference between the means of two sets of variables *which are not correlated* is given by the formula  $E_{1-2} = \sqrt{E_1^2 + E_2^2}$  it is apparent that the probable error of a difference ( $E_{1-2}$ ) between these means, when each has the same probable error, equals  $E\sqrt{2}$ . The values in the second vertical column of Wood's table give the ratios of the difference to such probable errors for the desired significance. For only a reasonable degree of assurance that conclusions as to the significance of an observed or hypothetical difference in an agricultural experiment are accurate, say 30 out of 31 times, the lower half of the table shows that the ratio of the difference ( $D$ ) between two variables, each subject to the same probable error, to the error of that difference,  $\frac{D}{E_{1-2}}$ , must equal 3.81.

Applying this method of reasoning to the present situation, with the probable error of a single plot ( $E_s$ ) noted above (10.99 pounds or 8.76 per cent), a difference of 3.81  $E_s = 41.87$  pounds per tree per plot (or 33.4 per cent of the mean tree yield) for six years would be necessary between any 2 plots to be certain 30 out of 31 times that a response to treatment in a given direction would be real.

Since it is often desirable to determine the effect of cultural treatments which are not expected to cause such great differences as those

<sup>7</sup> In the actual interpretation of the results of the future experiment by some of the methods which are to be discussed, the use of the normal distribution for the calculation of probabilities may not be warranted, owing to the small numbers of observations upon which the standard deviations may be based. Under such conditions Student's<sup>(57, 58)</sup> distribution of standard deviations of small samples should be used. Love,<sup>(29, 30)</sup> Love and Brunson,<sup>(32)</sup> Fisher,<sup>(12)</sup> McEwen,<sup>(34)</sup> Shewart,<sup>(52)</sup> and others have emphasized, extended, or facilitated the use of this distribution, while McEwen<sup>(34)</sup> and Conrad<sup>(9)</sup> have drawn attention to methods of comparing probabilities calculated from the two fundamental distributions. The theory of small samples has recently been summarized by Rider.<sup>(43)</sup> In the present study, however, the variability of the population is determined from a moderately large number of individuals, which may be considered to be normally distributed, as indicated above. Therefore, the calculation of the various statistical constants for individual observations and combinations of observations may be carried out according to the ordinary practices.

<sup>8</sup> This is the point of view assumed by Student<sup>(57)</sup> and by McEwen.<sup>(34)</sup> Of course, there are places where differences in either direction should be considered, and that part of Wood's table showing odds for them is included for reference.

noted above, it is apparent that the use of a single plot for each treatment is entirely unsatisfactory.

The difficulty obviously arises as a result of the failure of the yield of each plot to represent the yield of every other plot, or, in other words, to be a representative sample of the field as a whole. It is important that efforts be made to reduce the error of the individual treatment to smaller dimensions by securing a more nearly representative sample of the field for each treatment. Certain attempts to do this have been made.

#### THE EFFECT OF REPLICATION

It has been shown repeatedly that one of the most effective methods of reducing the error of the individual treatment is to increase the area devoted to it. The larger the area occupied by the sample used for each treatment, the more likely it is that its yield will represent that of the field as a whole. In a blank experiment, the hypothetical treatment area can be enlarged by increasing the number of unit plots assigned to each combination, or treatment, plot. The selection of the unit plots to be combined can be accomplished in many ways. It is possible, of course, to combine contiguous unit plots, but this has the effect of merely increasing the size of the single unit plot. It is also possible to select unit plots at random throughout the field and combine them into combination plots. With random choice of plots for this purpose an increase of the number of plots per combination should reduce the probable error of the combination plot ( $E_c$ ) approximately in the relation  $E_c = \frac{E_s}{\sqrt{n}}$  where  $n$  is the number of unit plots per combination, and  $E_s$  is the probable error of a single unit plot.

The effects of using various numbers of unit plots for each hypothetical treatment, with the replicates arranged in different ways, have been studied in the present experiment. Hypothetical combination plots, consisting of various numbers of unit plots, have been devised for each hypothetical treatment. In one set of comparisons the unit plots are contiguous, so that the entire area devoted to one combination plot is in one parcel.<sup>9</sup> In another set, the unit plots of the combination plot are systematically replicated, being separated by a number of units equal to the number of combination plots, less one. The coefficients of variation of combination plots of various sizes which have been formed in these ways are shown in table 17. In this table the theoretical coefficients

<sup>9</sup> In these arbitrary arrangements, plots were combined as follows: starting with plot D2 and continuing to D54, then from E2 to E24, from F32 to F54, etc., proceeding from north to south in each block, and from east to west between blocks. For ease of calculation each successive block is regarded as a continuation of the one before it. Plots which are not needed to make up complete combination plots at the south end of block M are disregarded.

which would be expected on the basis of random grouping of units of the combination plots are also given for comparison.

When contiguous plots are combined it is found that the variation of the combination plots is reduced as their size is increased, but that the reduction is not nearly so rapid as that expected theoretically on the basis of random replication. This is the result usually obtained by such grouping (Stevens and Vinall<sup>(55)</sup>). It is due to the fact that the yields of adjacent plots are positively correlated, in much the same way as are the yields of the trees in the same plots.

If, on the other hand, the area devoted to each treatment is increased by systematic replication of the unit plots, the variation of the combination plots is ordinarily reduced in somewhat the same degree as that expected by random replication (Stadler,<sup>(54)</sup> and Stevens and Vinall<sup>(55)</sup>). In the present case it is seen that the coefficients of variation of such combinations are, in the case of treatments of the same numbers of unit plots, slightly less than those anticipated according to the theory of probabilities.<sup>10</sup>

<sup>10</sup> It is desirable to know if the differences between col. 3 and cols. 4 and 5 of table 17 are significant. It has been pointed out to us by Professor G. F. McEwen, Scripps Institution of Oceanography, La Jolla, California, that, because of the approximate normality of the distribution from which the original standard deviation was derived, it is logical to determine the retical coefficient of variation for a small number of plots as in col. 3 of table 17, whereas the values in cols. 4 and 5 are subject to correction because of the small number of groups of plots ( $N$ ) (col. 2) from which these standard deviations are calculated. The value of the factor by which the standard deviation, or coefficient of variation, may be multiplied

to give an approximation to the true values, is  $\sqrt{\frac{N}{N-3}}$  for values of  $N$  greater than 30. For values of  $N$  equal to or less than 30, Dr. McEwen proposes the following method based on equating probabilities in Student's and the normal distribution.

Odds of 30 to 1 ( $P=0.967$ ) are frequently regarded as the threshold of significance; therefore for any desired value of  $N$  the value of  $Z$  corresponding to this probability, as given in Student's table, is multiplied by  $\sqrt{N}$  (since  $Z = \frac{x}{\sigma_x}$ ) to obtain  $Z\sqrt{N} = \frac{x}{\sigma_x}$  according to Student's distribution for small samples. The value of  $\frac{x}{\sigma_x}$  corresponding to the same probability is then found in a table of the distribution of the normal probability integral. McEwen's<sup>(34)</sup> tables 13 and 14 are very convenient for this purpose. The ratio  $\frac{Z\sqrt{N}}{\frac{x}{\sigma_x}}$  is now the correction factor by which the measure of variability of the small population is multiplied.

Applying these corrections to the coefficients of variation in the last two columns of table 17, and calculating the probable errors by the usual formula, the following values are obtained:

Number of plots ( $N$ )	Theoretical value ( $\frac{x}{\sigma_x} \div \sqrt{N}$ )	Correction factor for cols. 4 and 5 of table 17	Corrected coefficient of variation	
			Contiguous plots combined	Systematically replicated plots combined
	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
195	12.98 $\pm$ 0.45	—	12.98 $\pm$ 0.45	12.98 $\pm$ 0.45
97	9.18 $\pm$ 0.45	1.016	11.34 $\pm$ 0.56	8.01 $\pm$ 0.39
48	6.49 $\pm$ 0.45	1.033	10.13 $\pm$ 0.70	5.82 $\pm$ 0.40
32	5.30 $\pm$ 0.45	1.050	9.49 $\pm$ 0.81	3.85 $\pm$ 0.32
24	4.59 $\pm$ 0.45	1.072	7.91 $\pm$ 0.77	4.19 $\pm$ 0.41

From this tabulation it may be calculated that the *minimum* difference between the values of the theoretical coefficients and the coefficients of plots which are made up of contiguously combined units is 2.16 $\pm$ 0.72 per cent, which may be considered barely significant. There is a real difference, therefore, in variation of combination plots made up in these different ways. On the other hand, the *maximum* difference to be found between any of the theoretical coefficients and the coefficients of systematically replicated plots for any given number of plots is 1.45 $\pm$ 0.55, which is hardly considered significant. The variation of the systematically replicated plots, therefore, may be considered to approximate the theoretical.

The effect of the variability of the combination plots upon the difference theoretically necessary to give any desired degree of assurance that a real difference in yield would be caused by the treatments, can be calculated by the method discussed above. In order to obtain odds of 30 to 1 that the conclusions are sound, the probable error of a single

TABLE 17

EFFECT OF GROUPING VARIOUS NUMBERS OF UNIT PLOTS INTO COMBINATION PLOTS  
ACCORDING TO VARIOUS METHODS

Number of unit plots per combination ( <i>n</i> )	Number of combinations possible ( <i>N</i> )	Coefficient of variation ( <i>C</i> ) of combination plots, per cent		
		Theoretical = $C \div \sqrt{n}$	Contiguous plots combined	Systematically replicated plots combined
1	2	3	4	5
1.....	195	12.98	12.98	12.98
2.....	97	9.18	11.16	7.88
4.....	48	6.49	9.81	5.63
6.....	32	5.30	9.04	3.67
8.....	24	4.59	7.38	3.91

TABLE 18

THE DIFFERENCES NECESSARY BETWEEN THE MEANS OF COMBINATION PLOTS  
TO INSURE ODDS OF 30 TO 1 THAT THE DIFFERENCE IS DUE TO TREATMENT\*

(In percentage of the mean yield of combination plots)

Number of plots per combination	Theoretical, with random sampling	Contiguous plots combined	Systematic- ally repli- cated plots
1	2	3	4
1.....	33.36	33.36	33.36
2.....	23.59	28.68	20.25
4.....	16.68	25.21	14.47
6.....	13.62	23.23	9.43
8.....	11.80	18.97	10.05

\* In these calculations 195 plots were used.

combination plot in percentage ( $= \pm 0.6745 C$ , where *C* is the coefficient of variation, as given in table 17) should be multiplied by 3.81, if the direction is known or assumed. The results of this calculation are given in table 18.

The value of the use of systematically distributed unit plots for each treatment as compared to the use of the same number of contiguous unit plots is apparent from table 18. It may be seen that the minimum

difference necessary between combination plots, formed from as many as 6 or 8 systematically distributed units, would need to be about 10 per cent of the mean annual tree yield, to insure odds of only 30 to 1 that a difference in one direction would be due to factors other than chance. As shown by columns 2 and 4 of table 18, these figures are approximately those which would be expected on the basis of the probability theory. The fact that the difference with 6 unit plots per treatment is less than with 8 plots indicates that certain distributions were by chance very favorable ones.

The magnitude of the difference necessary to give statistical precision to the comparisons between combination plots which are arranged in these ways, and the necessity of using large areas of land for each combination plot, point to the desirability of reducing the chance errors of the field by other methods if it is possible to do so.

#### THE USE OF CHECK PLOTS

Variability in the yield of plots of a uniformly treated experimental field is due to two types of factors. Factors of the first type are accidental, and may occur at random throughout the planting. They should be maintained at a minimum level by great care in conducting the experiment. Factors of the other type are commonly called systematic errors and tend to make all or a part of the plots yield alike. Thus climatic conditions may tend to depress the yields of all plots in certain years, or they may affect the plots in certain areas of the field more adversely or favorably than in other years. It has been shown above that the difficulties encountered in endeavoring to obtain a fair sample of the effects of the annual variations in climatic conditions on trees are very great. An element of judgment enters into the decision as to the length of time an experiment, and especially a preliminary experiment, should be continued. It was shown, however, that the use of the mean yield of six years in the present case reduced the variations somewhat, and that it seems to give a fairly reliable index of productivity.

Variations in the fertility of the soil of a single field are in general of a systematic nature also, and have long been known to be very important. Although exceptions occur occasionally in the case of single plots in an experiment, Harris<sup>(18)</sup> and several others have demonstrated that the mean correlation coefficient between yields of contiguous plots is nearly always positive and significant. The data presented above for the yield of combinations of contiguous plots showed that such a correlation exists between yields of adjacent plots in the orchard under discussion, and that important systematic variations are involved.



An attempt to reduce the errors due to this type of variation and thereby make the experiment more reliable has frequently led to the distribution of check plots throughout the experimental field. These plots serve (1) as the basis of comparison with nearby test plots, or (2) as a means of correcting the yields of the test plots, after which the corrected yields of the various plots may be compared among themselves by common statistical processes. The effectiveness of these two methods of employing check plots has been studied with the present data.

However, it is doubtful if the results of such a study of uniformly treated material prior to the start of an experiment should be applied without some qualification. To do so would be to assume that the yields of check and test plots would be correlated exactly to the same extent with different treatments as they are under conditions of uniform culture. Such may not always be the case. Stadler,<sup>(53)</sup> for instance, found that in variety trials with small grains, the nature of the variety used as the check was a factor in determining the efficiency of adjustment of test-plot yields. This is a phase of the problem of orchard trials upon which insufficient information is available. It is possible that certain treatments in such experiments might occasionally alter correlations between yields of nearby plots, and either increase or decrease systematic variations as compared with those observed under uniform conditions of culture. Although this possibility should be kept in mind during the following consideration of the effect of the use of check plots for comparison and for adjustment, conclusions based upon blank experiments should be correct in most cases.

*Method of Calculating Adjusted Yield from Theoretical Check Yield.*—The process of correcting the yields of test plots is termed adjustment of yields. Ordinarily, the first step in the process, as Stadler<sup>(53)</sup> has explained, is the calculation of the theoretical check yield of each test plot, which is the probable yield of each test plot, provided it had been given the same treatment as that of the check plots. Various methods of arriving at this value have been proposed, based upon assumptions which must be made as to the nature of changes in natural productivity from one part of the field to another between check plots. The use of the uniformity trial makes it possible to determine the probable value of these assumptions.

The next step in adjustment of the yields of test plots consists in calculating from the theoretical check yield the hypothetical or adjusted yield of the test plot, i.e., the approximate yield that would have been obtained provided the plot had been one of *average* fertility.

There are several ways of making this approximation. The theoretical check yield ( $T_{ch}$ ) may be calculated by one of several methods which will be discussed. From this value, according to one method which seems to be useful for the present purpose, a factor, which Stadler<sup>(53)</sup> calls the "plot value" ( $P$ ) may be determined by use of the formula:

$$P = \frac{T_{ch}}{M_{ch}},$$

where  $T_{ch}$  is the theoretical check yield, and  $M_{ch}$  the mean yield of the check plots. The adjusted yield is then obtained by:

$$Y_{adj} = \frac{Y_{act}}{P},$$

where  $Y_{adj}$  is the adjusted yield,  $Y_{act}$  is the actual yield of the test plots, and  $P$  the plot value. The two formulas may be combined for ease in calculation. When yields of check plots are adjusted by these formulas their variability is eliminated, for in that case  $T_{ch} = Y_{act}$ , and hence  $Y_{adj}$  becomes equal to  $M_{ch}$ . If the actual yields of the test plots should equal their theoretical check yields, the variability of the adjusted yields of the test plots would also be eliminated. The reduction of the variation of test plots, as measured by the coefficient of variation, becomes, therefore, the measure of the efficiency of adjustment by this process.<sup>11</sup>

<sup>11</sup> Certain statistical relations are suggested by the formulas noted above. In the formula

$$Y_{adj} = \frac{Y_{act}}{P}$$

the adjusted yield is a quotient, or index, and the usual statistical formulas for such ratios (Yule, <sup>(70)</sup> p. 214) hold true. Thus the mean of the adjusted yields

$$M_{adj} = \frac{M_{act}}{M_{pr}} (1 - r V_{act} V_{pr} + V_{pr}^2).$$

In this formula,  $M_{adj}$  is the mean of the adjusted yields;  $M_{act}$ , the mean of the actual yields;  $M_{pr}$ , the mean of the plot values;  $r$ , the coefficient of correlation between either the plot values or the theoretical check yields and the actual yields of individual test plots;  $V_{act} = \frac{\sigma}{M}$  of actual yields; and  $V_{pr} = \frac{\sigma}{M}$  of either the plot values or theoretical check yields.

The coefficient of variation of the adjusted yields ( $C_{adj}$ ) can be calculated from their mean ( $M_{adj}$ ) and standard deviation ( $C_{adj} = \frac{\sigma_{adj}}{M_{adj}} \cdot 100$ ). Their standard deviation,  $\sigma_{adj}$ , is given by the formula (Yule, <sup>(70)</sup> p. 215) to which Stadler <sup>(54)</sup> has drawn attention:

$$\sigma_{adj} = \frac{M_{act}}{M_{pr}} (V_{act}^2 - 2r V_{act} V_{pr} + V_{pr}^2)^{\frac{1}{2}},$$

in which the symbols are the same as those given for the mean of the adjusted yields.

The coefficient of variation of the adjusted yields depends not alone on the correlation between the actual yields and either the calculated plot values or theoretical check yields, but also on the variability of actual and theoretical check yields. The correlation between actual yields and the calculated theoretical check yields of test plots is not, therefore, a precise index of the effectiveness of adjustment by means of check plots, although it has frequently been used for this purpose. This may be verified by observation of correlation coefficients calculated for the present data (table 19, col. 6).

*Effects of Various Methods of Calculating Theoretical Check Yield Upon Variation of Adjusted Yield.*—The effects of certain methods of estimating the yields of theoretical check plots from distributed check plots, and the effect of adjustment of the yields of test plots upon their variability, have been studied for the uniformly treated orchard under consideration with check plots at various intervals.

For this purpose, the rectangular section of the field consisting of blocks I to M inclusive has been studied, since this area offers possibilities of arrangement not possessed by the field as a whole. The data for the mean annual yield per tree for each plot for the period 1922–1927 have been used for this study. It is probable that conclusions drawn from a study of this part of the orchard may be approximately true for the entire field, since the coefficient of variation for the entire field is 12.98 per cent, while that for blocks I to M inclusive, is 13.15 per cent. No prominent differences in the frequency distribution of yields are apparent.

Five arrangements of check plots have been tried on the 108 usable plots of the 5 blocks noted. The essential difference between the arrangements is in the frequency of the check plots. In each case the end plots of each block consist of check plots.<sup>12</sup>

Four methods of comparison have been tried with each arrangement of check plots. These are based upon the assumption that the value of the theoretical check yield of each test plot is equal to: (1) the mean of all check plots; (2) the nearest check;<sup>13</sup> (3) the interpolated value found by assuming a constant fertility gradient between 2 check plots; and (4) the mean of the check plot mean and the interpolated yield.<sup>14</sup>

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<sup>12</sup> In two of the arrangements the number of test plots between check plots is not always constant, because the number of plots in the blocks is such that an extra plot is available with these two arrangements. In these two cases, therefore, the extra plot is arbitrarily inserted between the 2 check plots which are farthest south in each block, in order that the last plot may be a check plot.

The records of 2 plots in the field are not used, but the calculations are carried out in such a way that the interval is taken into account as if these 2 plots were used.

<sup>13</sup> When a test plot is located equidistant from 2 check plots their arithmetic mean is taken as the yield of the theoretical check.

<sup>14</sup> Various other formulas have been used by Stockberger,<sup>(50)</sup> Kiesselbach,<sup>(28)</sup> and others, which differ slightly from those given above in the assumptions involved in the calculation of the theoretical check yields. The above involve the basic assumptions of the majority of such formulas, however.

Methods of adjusting the yields of check plots before using them for adjustment of test plots have also been described. Thus Holtsmark and Larsen,<sup>(23)</sup> according to Roemer,<sup>(44)</sup> suggested that the mean of three nearby checks be obtained and used as a standard. McClelland<sup>(33)</sup> has also suggested that check plot values be smoothed by weighting them by a moving average method. It would seem that such operations occasionally might tend to produce lower correlations between theoretical check yields and actual yields of test plots if normal fluctuations in yields of contiguous test plots are rather sharp, and if checks were located at some distance. As abrupt changes in the yields of trees are frequently noted in the present experiment, in

Adjustment of the observed yields on the basis of these various calculations of the theoretical check yield was then made according to the method described in the text above.

The values for the coefficients of variation of the test plots before and after adjustment, according to the arrangement of each method, have

TABLE 19  
EFFECT OF ADJUSTMENT BY USE OF CHECK PLOTS UPON VARIATION OF TEST PLOT  
YIELDS, BLOCKS I TO M  
(Based on the mean annual yield per tree per plot, 1922-1927)

Frequency of checks	Method of calculating theoretical check yields	Coefficient of variation of test plots, in per cent		Reduction of variability, in per cent of unadjusted variability	Correlation coefficients between actual yield of test plots and their theoretical check yield or plot value
		Before adjustment	After adjustment		
1	2	3	4	5	6
No checks		13 15	.....	.....	.....
3	1. Mean of checks.....	12 79	12 79	0 0	.....
	2. Nearest check.....	12 79	10 40	18 7	+0 688±0 042
	3. $2/3 C_1 + 1/3 C_2$ .....	12 79	9 08	29 0	+0 715±0 040
	4. $1/2 (C_m + 2/3 C_1 + 1/3 C_2)$ .....	12 79	9 46	26 0	+0 747±0 035
5 or 6	1. Mean of checks.....	13 26	13 26	0 0	.....
	2. Nearest check.....	13 26	11 44	13 7	+0 599±0 048
	3. $4/5 C_1 + 1/5 C_2$ .....	13 26	9 53	28 1	+0 590±0 048
	4. $1/2 (C_m + 4/5 C_1 + 1/5 C_2)$ .....	13 26	10 52	20 7	+0 599±0 048
7	1. Mean of checks.....	12 88	12 88	0 0	.....
	2. Nearest check.....	12 88	11 59	10 0	+0 586±0 048
	3. $6/7 C_1 + 1/7 C_2$ .....	12 88	10 06	21 9	+0 641±0 042
	4. $1/2 (C_m + 6/7 C_1 + 1/7 C_2)$ .....	12 88	9 51	26 2	+0 643±0 042
10 or 11	1. Mean of checks.....	12 85	12 85	0 0	.....
	2. Nearest check.....	12 85	12 11	5 8	+0 620±0 043
	3. $9/10 C_1 + 1/10 C_2$ .....	12 85	10 54	18 0	+0 636±0 042
	4. $1/2 (C_m + 9/10 C_1 + 1/10 C_2)$ .....	12 85	9 82	23 6	+0 662±0 040
21	1. Mean of checks.....	12 87	12 87	0 0	.....
	2. Nearest check.....	12 87	11 99	6 8	+0 612±0 043
	3. $20/21 C_1 + 1/21 C_2$ .....	12 87	8 38	34 9	+0 721±0 033
	4. $1/2 (C_m + 20/21 C_1 + 1/21 C_2)$ .....	12 87	9 53	26 0	+0 693±0 035

been computed and are given in columns 3 and 4 of table 19. The percentage reduction in variation, based upon the coefficient of variation of the unadjusted yields, is given in column 5.

It was found by inspection of the formulas for the plot values and for the adjusted yields that when the theoretical check yields of the test plots are assumed to be equal to the mean yield of the checks, no change in variation of the test plots occurs by adjustment with the method used.

common with many other uniformly treated orchards, the use of these methods in experiments with such material is probably of doubtful value as compared with the simpler formulas.

Although this method of comparison is frequently used in some form, it is apparent that adjustment based upon it must be differently applied, if it is to be helpful.

If the theoretical check yield of the test plot is taken as equal to the yield of the nearest check, and the actual yield of the test plot is adjusted accordingly, a slight to moderate reduction in variability is effected. This reduction occurs with checks at all the intervals tried, but it is greatest with the checks at close intervals.

The interpolation formulas (numbered 3 in tables 19 and 20) are based upon the assumption that the normal productivity between check plots varies uniformly. In the formulas  $T_{ch} = \frac{2}{3} C_1 + \frac{1}{3} C_2$ , etc.,  $C_1$  is the yield of the nearest check on one side of the test plot, and  $C_2$  the yield of the nearest on the other side. Adjustment of the observed yields on the basis of the theoretical check yields obtained by this calculation results in values for the adjusted yields of the test plots which show considerably less variation than do the actual yields. The amount of reduction is greatest when the checks are close together and decreases as the interval between checks is increased, except at the greatest interval, with the checks only at each end of the field. Although the variation is reduced most with this extreme interval, indicating in this particular field a grading in productivity from one side to the other, certain very serious objections to locating the checks at this interval will shortly be noted.

If the theoretical check yield is assumed to be the mean of the check mean and the interpolated yield (formulas numbered 4 in tables 20 and 21), a reduction of the variation is also obtained by adjustment. In this case the amount of the reduction is about the same regardless of the interval between checks. However, it is apparent that the use of the check mean as a factor in calculating the theoretical check yields in formulas numbered 4 has not consistently changed the effect of adjustment as compared with that resulting from adjustment based upon the use of the interpolated yield alone as the theoretical check yield.

From the data of table 19 it appears that formulas involving the use of the interpolated yield, either alone or in conjunction with the mean of all the checks, in the calculation of the theoretical check yield give adjusted yields of less variation than any of the other methods tried.

*Effects of Various Frequencies of Check Plots Upon Variation of Adjusted Yields.*—In regard to the location of checks at various frequencies, if the case in which checks were located at every twenty-first plot is disregarded for the moment, it appears that the average reduction of variability of adjusted yields obtained by all methods is greatest

when the checks are located at intervals of 3 plots. The average reduction becomes slightly less with checks at more remote intervals. This decrease in efficiency with various checks located at progressively greater distances is regularly reflected when the theoretical check yield is calculated on the basis of the nearest check (formulas numbered 2), and on the basis of the interpolated yield between check plots (as in formulas numbered 3). It is not apparent when the mean of the check mean plus the interpolated yield is used (formulas numbered 4).

It was observed that the greatest reduction in variation was obtained by adjustment with the grading, or interpolation, method of calculation when checks were located at every twenty-first plot, indicating a gradual change in fertility from one side of the field to the other. In this case the check-plot rows were separated by a distance of 1.056 feet, and were located at the extreme northern and southern edges of the field. The value of such an arrangement may be seriously doubted. It may be a characteristic of this particular orchard due, by chance, to favorable yields of the limited number of check plots, which could not be reasonably expected to occur frequently. In addition it is conceivable that fluctuations might occur over a long period of years in plots distantly removed from the check, which will be independent of the treatment. The theoretical check yield would not reflect this change. A fundamental conception underlying the use of check plots, namely, that a correlation exists between the theoretical check yield and the actual yield (Richey,<sup>(40)</sup> and Stadler<sup>(54)</sup>), would be violated in such a case.

An additional hazard would result from the location of the check plots at the extremities of the field, or at extremely long intervals. This is the consequence resulting in case one check plot is rendered unfit by accident for comparison with the test plots. Such a situation is much more likely to occur with plots near the edge of the field, particularly if they are ever subject to wind damage or to increased difficulty of heating during cold weather. The location of check plots at these points, therefore, does not seem to be warranted in any trials upon this orchard in spite of the favorable coefficients of variation of the adjusted test plot yields obtained by their use.

*Effects of Various Methods of Adjustment Upon Differences Necessary for Significance.*—In order to be significant for comparison between plots, it is ordinarily considered that differences in yield should be great enough to insure odds of 30 to 1 that they are not due to chance variations. The several methods of calculating the theoretical check yields used resulted in reducing in varying degrees the differences between adjusted yields which are required for this significance, as is

shown in columns 5 and 6 of table 20. The differences are expressed as percentages of mean adjusted yield of the test plots (this mean approaches the mean yield of check plots). These data were obtained by the procedure outlined in a preceding section of this paper. The minimum difference (in a given direction) between single unit plots found

TABLE 20  
EFFECT OF ADJUSTMENT OF TEST-LOT YIELDS BY USE OF CHECK PLOTS UPON  
DIFFERENCES NECESSARY FOR SIGNIFICANCE OF 30 TO 1 BETWEEN  
INDIVIDUAL TEST PLOTS AND BETWEEN THEORETICAL  
COMBINATION PLOTS\*

Frequency of checks	Number of unit test plots available	Theoretical number of unit plots for each of 15 combinations	Method of calculating theoretical check yield	Necessary differences in one direction, in per cent of mean adjusted yield of test plots		
				Between single plots		Between combination plots after adjustment
				Before adjustment	After adjustment	
1	2	3	4	5	6	7
No checks	108	7 20		33.79	.....	12.59
3	68	4 53	1. Mean of checks.....	32.87	32.87	15.44
			2. Nearest check.....	32.87	26.73	12.56
			3. $2/3 C_1 + 1/3 C_2$ .....	32.87	23.33	10.96
			4. $1/2 (C_m + 2/3 C_1 + 1/3 C_2)$ .....	32.87	24.31	11.42
5 or 6	83	5 53	1. Mean of checks.....	34.08	34.08	14.49
			2. Nearest check.....	34.08	29.40	12.50
			3. $4/5 C_1 + 1/5 C_2$ .....	34.08	24.49	10.41
			4. $1/2 (C_m + 4/5 C_1 + 1/5 C_2)$ .....	34.08	27.03	11.49
7	88	5 87	1. Mean of checks.....	33.10	33.10	13.66
			2. Nearest check.....	33.10	29.78	12.29
			3. $6/7 C_1 + 1/7 C_2$ .....	33.10	25.85	10.67
			4. $1/2 (C_m + 6/7 C_1 + 1/7 C_2)$ .....	33.10	24.44	10.09
10 or 11	93	6 20	1. Mean of checks.....	33.02	33.02	13.26
			2. Nearest check.....	33.02	31.12	12.50
			3. $9/10 C_1 + 1/10 C_2$ .....	33.02	27.09	10.88
			4. $1/2 (C_m + 9/10 C_1 + 1/10 C_2)$ .....	33.02	25.24	10.14
21	98	6 53	1. Mean of checks.....	33.07	33.07	12.94
			2. Nearest check.....	33.07	30.81	12.06
			3. $20/21 C_1 + 1/21 C_2$ .....	33.07	21.54	8.43
			4. $1/2 (C_m + 20/21 C_1 + 1/21 C_2)$ .....	33.07	24.49	9.58

\*Calculated from data of table 19.

necessary for significance is 23.33 per cent of the mean with checks at all frequencies, except at every twenty-first plot. This is a considerable reduction from the differences necessary between unadjusted yields of unit plots, which were found to be about 33 per cent.

Further decreases in the differences necessary between treatments could be obtained by increasing the area devoted to each treatment. If

this were done by random replication of the unit plots for each combination, or treatment, plot, it is probable that the coefficient of variation of the treatment, and hence the differences necessary for any degree of significance between treatments, would be decreased approximately according to the formula:

$$C_c = \frac{C_u}{\sqrt{n}},$$

where  $C_c$  is the coefficient of variation of the combination plots,  $C_u$  is the coefficient of variation of the unit plots, and  $n$  is the number of unit plots in each treatment. This increase in number of plots per treatment would result in increased reliability in this case, in the same way that it was shown to have an effect in the above discussion of the probable error of a single plot, and would be very effective in reducing the differences between combination plots necessary for significance.

The use of systematically replicated check plots as a basis for adjustment of test-plot yields in an experiment upon this orchard would apparently result in a reduction of the errors involved in trials upon it. However, such a decrease would be obtained only by the allocation of a large number of plots for use as check plots which might otherwise be used as additional replicates of the test plots. The possibility of securing greater reliability by the use of check plots as described, than by increasing the area of each treatment by the use of the possible check plots for additional replications of combination plots may be determined in a theoretical manner.

If 15 treatments are to be tried upon the plots of blocks I to M inclusive, it is possible to determine the probable error of each combination plot, based upon the variation of the adjusted yields where check plots are used, and upon the variation of all of the plots for the unadjusted yields. The total number of test plots available with each arrangement of check plots varies, of course, and the theoretical (since fractions are involved) number of these which may be used for each of the 15 treatments is given in columns 2 and 3 of table 20. Since, in a case of random assortment, the coefficients of variation of unit plots bear the relation noted above to the coefficient of variation of combination plots, it is possible from these data to calculate the probable error of a single combination plot, and, hence, the difference between combinations or treatments necessary for significance. The values necessary to assure odds of 30 to 1 are set forth in column 7, for assumed differences in one direction. If the arrangement of checks at each side of the orchard (every twenty-first plot) is disregarded, the adjustment of yields by check plots does not reduce the difference necessary for significance of this



degree by more than 2.5 per cent of the mean yield (from 12.59 to 10.09 per cent), a reduction in percentage of 19.86 per cent, based upon the unadjusted yields where no checks are used.

Judged by this method of analysis, the interpolation formula, 3, is very slightly more efficient than the combination formula, 4, with check plots at intervals of 3, and 5 or 6 plots. With checks at intervals of 7, and 10 or 11 plots the combination formula shows a very slight advantage over the interpolation formula. The calculation of the theoretical check yield by either of these formulas is apparently more reliable in this comparison than according to the assumption that the theoretical check yield of each test plot is equal to that of the nearest check. Although the illustration is hypothetical, since the number of unit plots per treatment has been expressed in fractions, it indicates that adjustment of yields by means of checks would probably reduce the errors of trials upon this orchard more than would the increased replication of test plots that would be made possible by elimination of the check plots. Although the increase (nearly 20 per cent) in reliability seems important, the minimum differences between combinations which would be necessary for only moderate significance (10.09 per cent of the mean adjusted yield), are still greater than some of the treatments may cause. It is desirable to see if even better methods of comparison may be obtained with the use of check plots.

*The Comparison of Single Treatments With a Check Treatment.*—The elimination of systematic errors in various parts of the experimental field has been attempted frequently by the comparison of the various treatments with a standard treatment which is used for the check plots. In such cases the treatment of the check plots is ordinarily one which is known to give good results, and which it is desirable to surpass. The method involves, fundamentally, the calculation of the difference between the mean yield of each treatment and the mean of its theoretical check yields, which is then compared with the probable error of the difference as a test of significance. The probable error of a series of differences can be determined directly, or, if certain statistics are known, from the full formula for the variance (squared standard deviation) of a difference. The latter formula, as frequently emphasized (Student,<sup>(59, 60)</sup> Kemp,<sup>(25)</sup> Richey,<sup>(40)</sup> Sax,<sup>(47)</sup> etc.) is:

$$\sigma_{1-2}^2 = \sigma_1^2 + \sigma_2^2 - 2r_{12} \sigma_1 \sigma_2$$

The last term in the equation can be eliminated only in case both populations are sampled at random, when  $r = 0$ . This is obviously not the case in the present situation, for the theoretical and actual yields of the test plots are correlated.

Another method for obtaining the differences necessary for comparing a single treatment with the check treatment leads to the same results as the methods just given, and the calculations may sometimes be more conveniently made. This involves the determination of the differences between the mean of the check plots and the adjusted yields of the test plots devoted to the particular treatment, when adjustments are made according to the method previously discussed. The calculation of the variance of the differences, as determined by means of the complete formula given above, is very simple in this case, for the variance of the mean of the checks is zero, and there is no correlation between the mean of the checks and the adjusted yields. Hence (Stadler<sup>(54)</sup>), the variance of the difference equals the variance of the adjusted yields.

The relations of these two methods may be illustrated by the case given above, with a check every third plot when the calculation of the theoretical check yield is carried out according to the formula  $\frac{2}{3} C_1 + \frac{1}{3} C_2$ . The following values have been determined:

$\sigma_1$ , the standard deviation of theoretical check yield = 13.2

$\sigma_2$ , the standard deviation of actual yields = 15.74

When these values are substituted in the full formula for the standard deviation of a difference, we find:

$$\sigma_{1-2} = 11.18$$

This value, when divided by 123.25 (the mean of the adjusted yields) and multiplied by 100 to place it on a comparable basis, becomes 9.07 per cent, which is very close to the value given in table 19 for the coefficient of variation of the adjusted yields of the test plots (9.08 per cent). The choice of method depends solely on convenience.

The use of the value 9.07 per cent as the coefficient of variation of a difference, makes it possible to calculate the difference in yield necessary for significance between any 2 theoretical check and test plots. In this case column 1 in the table of odds given in Appendix A should be used since the difference is compared with its own probable error. In order to obtain odds of 30 to 1 that conclusions as to the significance of a given difference ( $D$ ) are correct, then,

$$\frac{D}{0.6745 \cdot 9.07} = 2.70; D = 16.52 \text{ per cent.}$$

By comparison with comparable data from table 20, the differences necessary for significance between single plots may thus be seen to be considerably less when comparisons are drawn between individual theoretical check plots and test plots, than when they are drawn between

different individual adjusted test plots (23.33 per cent). This is in agreement with Stadler's discussion. <sup>(54)</sup> The replication of such plots should reduce the errors by approximately  $\frac{1}{\sqrt{n}}$ , where  $n$  is the number of test plots per combination plot.

The use of methods of differences between test and check plots, however, does not appear to be an advantage when extended to a comparison of 2 test plots or 2 combination plots (test plots receiving the same treatment). In order to compare the mean differences and their probable errors, which have been determined for each of the combination plots in comparison with their check plots, the formula for the probable error of a difference between two means must be used. In that case the use of methods of differences between combination and check plots gives the same results as the direct comparison of adjusted yields of the test plots.

#### THE COMPARISON OF NEARBY TEST PLOTS BY METHODS OF DIFFERENCES

It has been indicated above that in the full formula for the variance of a difference,  $\sigma_{1-2}^2 = \sigma_1^2 + \sigma_2^2 - 2r_{1-2} \sigma_1 \sigma_2$ , the last term can be eliminated only when the correlation between the observations is zero. In field trials this is seldom the case, for there is usually a tendency towards concomitant variation of nearby test plots between which comparisons are drawn. Therefore, it is possible to reduce the error of the mean difference between 2 treatments, as usually determined, by the calculation of the correlation between nearby test plots of the 2 treatments and the utilization of the last term in the equation. Love<sup>(29)</sup> has pointed out that the same effect can be secured by obtaining the mean of the differences between individual correlated test plots which are being compared, and directly calculating the variance of that mean.

This procedure has the effect of reducing the errors due to systematic variation, which is the cause of the correlations. The amount of the reduction will depend upon the size of the correlation. This consideration has led Student,<sup>(59)</sup> p. 273, to remark that "the art of designing all experiments lies even more in arranging matters so that  $r_{1-2}$  is as large as possible, than in reducing  $\sigma_1^2$  and  $\sigma_2^2$ ."

It is anticipated that the results obtained on the present field by the application of the method of comparing the unadjusted yields of test plots which are replicated at random would, on the whole, be somewhat less reliable than those obtained by the use of check plots for adjustment. Because of the large number of treatments which are proposed for trial the plots to be compared in the same replication series would be located, on the average, at some distance from each other. In some cases they would be at opposite sides of the field in different blocks.

Consequently, the correlation between "paired" test plots would be small.

The calculation of the errors of all possible differences between treatments is very laborious. Fortunately, Student<sup>(59)</sup> and R. A. Fisher have developed a method by which the mean error of the differences of all the possible comparisons can be obtained. This method may be used to illustrate the effect of the procedure upon the material under consideration. By their formula, the variance of a mean difference between any 2 sets of  $m$  treatments, each tried  $n$  times, is:

$$\sigma_d^2 = \frac{2m(\sigma_T^2 - \sigma_R^2 - \sigma_G^2)}{(m-1)(n-1)},$$

when  $\sigma_T^2$  is the total variance of the plots;  $\sigma_R^2$ , the variance of the treatment means; and  $\sigma_G^2$ , the variance of the replication series means. The formula is discussed in some detail by Student,<sup>(59)</sup> and in a slightly modified form by Engledow and Yule.<sup>(11)</sup>

The application of the formula to the data at hand can be made by assuming that any likely number of plots be devoted to each treatment on the 108 plots in blocks I to M inclusive. If 4 plots are used for each theoretical treatment, 27 treatments are possible on this area. If the treatments in each replication series are in the same order, starting at I-2 and progressing to I-44, then to J-2, etc., repeating until the 27 treatments have been allocated to 4 plots each, the calculation of the formula indicates that the variance of the mean difference,  $\sigma_d^2$ , between the means of the treatment yields is 132.26, and the standard deviation of the mean difference,  $\sigma_d$ , is, hence, 11.50 pounds; this is 9.48 per cent of the mean yield of the 108 plots. If this percentage be multiplied by 0.6745 to obtain the probable error in per cent and by 2.7 (the ratio of the mean to its probable error necessary to give 30 to 1 odds), the difference between treatment means necessary to give this significance is found to be 17.26 per cent. This difference is not far from those which were indicated in table 18 as theoretically necessary between the means of groups of 4 test plots systematically replicated throughout the entire field. Although somewhat better arrangements than the one noted might be obtained by chance, it seems probable that, with many treatments and few replications on this planting, the desired high correlation between the plots in each replication series would be lacking. Consequently, with chance or systematic arrangement of plots, the comparison of nearby test plots by methods of differences would not appreciably eliminate the effects of systematic soil variations nor reduce the errors of experiments upon the planting.

#### ADJUSTMENT OF YIELDS OF TEST PLOTS BY MEANS OF CONTIGUOUS TEST PLOTS

Various investigators have attempted the elimination of systematic variations in different parts of the experimental field by the use of the yields of neighboring test plots as the standard for the adjustment of the individual plots. The methods of Hummel,<sup>(24)</sup> Mitscherlich,<sup>(35)</sup> Surface and Pearl,<sup>(61)</sup> and Richey<sup>(40, 41, 42)</sup> are perhaps best known. Their general advantages have been discussed by Stadler,<sup>(54)</sup> and consist in the release of check plots for increased replication of test plots, the avoidance of the possibly unfair effect of adjustment of certain treatments by means of a single check treatment, the avoidance of undue effect of great chance fluctuations in the yield of a particular plot which may be used as a check, and the determination of the theoretical check yield of the test plot by means of contiguous plots rather than by remote plots.

The objections to such methods of adjustment in the usual type of cultural trials with fruit trees lie, however, in the probability that large numbers of treatments must be tried in a single experimental field, and that the number of replications will be narrowly limited. In this case a fundamental conception upon which certain of the above methods are based, may be violated, namely, that the mean yield of each set of replicated test plots designated as a combination plot is a "fair index of its productiveness" (Richey<sup>(40)</sup> p. 90). Furthermore, unless the number of replications of each treatment equals or exceeds the number of treatments in the experiment, the use of these methods assumes (Richey<sup>(40, 41)</sup>) that the influence of particular treatments upon the theoretical check yields of plots contiguous to them will not be so great as to introduce serious errors. In orchard trials of long duration, the range of the responses caused by cultural treatments may be extremely wide, so that the effects of adjustment by plots differing widely in yield, might unduly favor or handicap individual plots.

These considerations make it evident that if a trial which involves many widely differing treatments with relatively few replications for each is anticipated, the study of the effect of adjustment of yields of test plots by one another while under conditions of uniform culture prior to the start of the experiment proper, may occasionally lead to expectations which will not be justified after the experiment goes into operation. Fortunately the requirements of such methods as to the plan of the experiment in addition to those given above are not stringent, being only that the treatments should not appear in the same order in the different

replication series. As a consequence the methods may often be applied and their benefits realized whether check plots are employed or not.

It is apparent that in the plan of the experimental orchard under discussion it would be unwise to place the entire responsibility for the reduction of the experimental errors and for the interpretation of the results to be obtained upon such methods of adjustment of yields. It would seem safer to find the alternative plans which appear to be most reliable for the conditions of the experiment. The application of methods of adjustment of yield data by means of contiguous test plots may still be made when the experiment is in operation, and used in the interpretation of the effect of the treatments, if they seem to lead to more reliable conclusions than other methods.

#### ADJUSTMENT OF YIELDS ON THE BASIS OF PAST PERFORMANCE

It has been observed in a previous section that in general a significantly positive correlation exists in successive years between the yields of plots planted to annual crops. It is conceivable, therefore, that the yields of the plots obtained under conditions of uniform culture might be used to establish indexes which will approximate the relative productive capacity of the plots. These indexes might then be used for adjustment of yields to reduce the effects of systematic soil variations, or as a basis for locating the plots for each treatment in the experiment.

Such a study involving both possibilities was made many years ago by Wagner.<sup>(62)</sup> He concluded that the relative yields of plots in two consecutive years are not sufficiently alike for their use to be of value. Other workers (Roemer<sup>(44)</sup>) with field crops have come to the same conclusion. However, their studies have been made upon the yields of single or of few years, rather than yields of a large number of years. As Lehmann,<sup>(27)</sup> Harris and Seofield,<sup>(18, 19)</sup> and others have shown, weather conditions exert a great influence on relative yields of plots planted to annual crops, and it is possible that other results might ensue if representative samples of the climatic effects could be obtained in studies of these methods. It also seems possible that methods of analysis may be used which will take advantage of interannual correlations to reduce variability, even though the correlations may be small.

There are certain factors inherent in experimental work with trees which strongly suggest that the use of past records in formulating the plan of an experiment may be of more value than is the case with annual crops. The condition of each tree at the beginning of an experiment is the result of its parentage and environment, by which the size, nature of development, and state of vigor of the plants at the beginning of the experiment are established, and to some extent influenced during the

experiment. These characteristics are reflected in their yields. It is conceivable, therefore, that significantly positive interannual correlations may be found more consistently in the case of yields of trees than with annual crops where, in experimental field work, individual plant variations are ordinarily eliminated and the response of the plots depends only on the conditions of soil and climate prevailing in the particular crop year.

Attention has already been called in the present paper to the fact that the yields of fruit trees and plots of trees in individual plantings have frequently exhibited a tendency to have about the same gross variation in different years. This seems to imply a correlation in yields of trees in different years. Such correlations have, in fact, been observed by several writers.

The practical use of these tendencies of trees and of plots of particular plantings to maintain their variability and relative productivity over a term of years in the technique of orchard experimentation has been advanced by numerous investigators. Batchelor and Reed<sup>(4)</sup> discussed the suggestion that a knowledge of the variation of the trees of a planting, obtained under conditions of uniform culture, be used to estimate the errors of the future experiment. The suggested use of yield data secured prior to the beginning of the experiment has been carried further by Chandler<sup>(6, 7)</sup> who discussed the suggestion that the orchard be maintained under uniform care for a period of two to four years. Plots could then be so arranged that the average yield for each plot during this preliminary period would be nearly the same<sup>(6)</sup> p. 238,<sup>15</sup> "or else the yield of each plot during this preliminary period could be

<sup>15</sup> This procedure is similar to the technique of Wagner,<sup>(62)</sup> who selected for comparison plots which deviated equally from the mean yield in one year under conditions of uniform treatment. The following year Wagner compared the deviations of these paired plots under the same cultural conditions and found them unequal. Wagner also suggested that the deviations observed in a preliminary trial could be used for correcting the yields in the experiment which followed.

The method of combining plots so that the preliminary yields for each treatment were equal was followed by Lehmann.<sup>(27)</sup> This investigator kept a record of the yield of 105 untreated plots of Ragi or African millet, *Eleusine coracana*, from 1905 to 1907. At the end of this period he discarded the yields of 1906 because of extreme weather conditions, and established the relative yield of the plots in percentage of the mean, a process which he called "standardization." He discarded the plots which showed results widely divergent from the mean yield in 1905 and 1907 and averaged the yields of each of the remaining plots for these two years. These plots were then grouped, 2 plots to a treatment, in such a way that the mean yields of the combination plots were equal to the mean of the field for the preliminary period. No published results of the experiment as planned by Lehmann have been found. His experiment was carried on for a period of five years from 1908 by L. C. Coleman and then abandoned, since it was thought that "this sort of experimental procedure would not lead to accurate results. . . . The reason for this lay . . . in the fact that duplicate plots lay widely scattered on land lying on a fairly steep slope, so that soil moisture conditions were likely to vary widely. These depend upon a rainfall distribution that varies so from year to year that it would take probably ten or fifteen years to get a fair average." (Correspondence of L. C. Coleman dated May 31, 1929.)

used in weighting the results obtained from the plots receiving the different treatments."<sup>(7)</sup> p. 8.

The above suggestions concerning the application of preliminary yield records in the plan of the experiment seem to involve two fundamental concepts: (1) that by selecting replicates for all individual treatments so that the total preliminary yield may be about equal for each treatment, a more representative sample of the field may be obtained with the same number of replicated plots; (2) that the relative yield of each plot obtained under conditions of uniform culture, if expressed as a decimal fraction of the mean yield of the field, may be used as a plot value to adjust actual yields and to reduce variation in the experiment which follows. If the plot value remained constant during both the preliminary and actual trials adjustment based on plot values obtained in the period of preliminary testing would eliminate variation during the period of differential treatments. However, since the interannual correlations in yield have in no case been reported as equal to  $\pm 1.0$ , the plot values cannot be expected to remain constant. There is an error attached to their use which should be taken into consideration. The ordinary methods of standardization neglect this error.

However, another method of using preliminary yields to reduce the errors of an experiment may be employed. This method is based upon the calculation of the significance of the differences between correlated replicates of 2 treatments. The variance of the difference is decreased as the correlation between paired plots increases. In ordinary practice the contiguous plots are regarded as being in the same replication series for purposes of comparison, since there is frequently a positive correlation between nearby plots, as Harris,<sup>(16, 17)</sup> has demonstrated. It has been demonstrated heretofore, however, that when this method is applied to these data, using few replications which are widely separated, there is, on the average, no increase in reliability. This, it has been shown, means that there is little average correlation between the plots of the same replication series under such conditions. If, however, the plots of 2 treatments could be chosen for comparison on any other basis so that their yields would be correlated, the method of differences could be used to advantage. It seems probable that the relative yields of plots obtained prior to the beginning of an experiment could be used for this purpose. Richey,<sup>(41)</sup> p. 1163, in a discussion of this subject as applied to a hypothetical example with fruit trees, states: "if . . . . data obtained on the yields of the same plats under uniform treatment prior to beginning the experiment had shown the inherent productive capacity of the plats to be in this (or any other) order, it would be very desirable to pair the



plats as indicated by the preliminary data."<sup>16</sup> Recently Hoffman<sup>(22)</sup> has reported the plan of a fertilizer experiment with raspberries, based upon this principle. He selected plots for each treatment in such a way that the paired replicates were from the same yield group, as determined by the yields of the plants during the year 1929 under conditions of uniform culture.

The special advantages of such an arrangement would seem to persist as long as the yields of the plots, after the differential treatments have gone into effect, are correlated with the yields during the prior period of uniform care. As long as this correlation persists there would be some correlation on the average between the individual plots in each yield group.

A scheme in orchard experimentation which is fundamentally very similar was followed in 1924 by Anthony.<sup>(23)</sup> Waring<sup>(23)</sup> had previously reported that in several experimental apple orchards the yield of trees was correlated with the circumference of the trunk of the tree. Anthony<sup>(23)</sup> therefore, selected individual paired trees in a twenty-year-old orchard for different treatments on the basis of their equality in trunk circumference. He then planned to compare these paired trees by a method of differences. Since girth at the time the experiment is started is the result of growth preceding that time, and Waring had shown that girth is correlated with yield, the method is similar to that involving the pairing of plots on the basis of past yields.

If the preliminary yields of trees and plots of trees are correlated with their yields during an experiment, an advantage additional to those mentioned can be secured by arranging the plots on the basis of their preliminary yields. This is the possibility of obtaining for each treatment, samples of the trees (and plots) which are fairly representative of the variability of the experimental material. By this is meant the opportunity of selecting plots supporting trees in different conditions for each treatment. It is possible that some treatments might affect trees in all conditions of vigor which prevail in the plantings, while others may be effective only on trees relatively low in vigor. Such relations would be of great interest. In case a treatment does affect plots of the several yield groups differently, the correlation of the yields before and after treatment would probably be changed by that par-

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<sup>16</sup> A method of arrangement of the experimental orchard under consideration, in such a way that the mean preliminary yields of all treatments would be equal, and in which the plots of each treatment were arranged in the order of productiveness so as to permit the use of Student's<sup>(59)</sup> method for the determination of the mean variance of the difference between treatments was suggested by Mr. Richey in correspondence with the authors dated May 28, 1925.

ticular treatment from the relation that would have prevailed had the original cultural conditions been maintained. It is difficult to tell beforehand, however, whether alterations in this correlation would greatly change the differences necessary for significance in the actual trial. The coefficient of variation of the combination plots would also be altered and very possibly in the same direction as the change in the correlation coefficient. In any event the inclusion of the same number of comparable trees in each combination plot would seem desirable from a horticultural, as well as from a statistical point of view.

### PLAN OF THE EXPERIMENTAL ORCHARD

The present experiment with Washington Navel orange trees has been planned in an attempt to make use of any benefit which may accrue through study of the yields prior to the beginning of the differential treatments. This plan was placed in operation during the spring of 1927.

Sole dependence, however, upon methods of interpretation of the future experiment based upon standardization processes, would, with our present knowledge, involve certain dangers which have not been disregarded. Chief of these, even if the climate be adequately sampled, is that the correlation of yields before and after the beginning of the treatments may not be high enough for direct adjustment to be effective. This might be due to increasing age of the trees, which might influence their reaction to a given set of conditions. A likely source of disturbance, also, is the possibility that soil changes, either natural or because of treatment, may be progressive and that their effects may be cumulative either in the soil or in the trees. Under such conditions yields of the trees would reflect the changed conditions. It is felt, therefore, although the preliminary data may be valuable in the present case, that precautions should be taken to make possible the use of other methods of analysis than those based upon it.

### NUMBER OF REPLICATIONS

It was pointed out above that increasing the number of plots devoted to each treatment increases the likelihood that a representative sample of the field will be obtained for each treatment. When the replicates were systematically distributed in blocks I to M inclusive, the reduction in variability obtained by replication was approximately that expected on the basis of random sampling. Although it would be desirable to have a large number of replicates for each treatment it is nearly always

necessary to limit the number. Since the number of treatments which it is necessary to try in the present experiment is large, the number of replicates for each treatment must be small. The most practical use of the area for the present purpose appears to result when 4 plots are used for each treatment. This number of plots was found to be satisfactory by Batchelor and Reed<sup>(4)</sup> in their study of orchard uniformity trials. It has also been found of satisfactory reliability by Livermore<sup>(28)</sup> for potato trials with single-row plots 36 feet long, the variability of which is comparable to that of plots in orchard trials.

This limitation of the number of replications lessens the accuracy of interpretation of the experiment. However, it is believed that the loss in accuracy due to this factor will be compensated by the cumulative effect of the treatments. There is also the possibility of interpreting the future experiment by means of pairing plots in this experiment. This possibility is enhanced by the fact that the specific treatments have been planned in such a way that in many cases a larger number than 4 plots may be compared. These possibilities, however, depend on the nature of the treatments and cannot enter into the present discussion.

The reliability of comparison between any 2 treatments, each repeated 4 times on plots which are not correlated in yield, can be approximately determined. If the coefficient of variation of the plots is the same during the period of differential treatments as before their initiation (see p. 114), average differences of approximately 16.7 per cent would be necessary between 2 treatments. If the variation can be reduced by any method, however, the differences necessary for significance will be decreased proportionately.

#### THE ALLOCATION OF CHECK PLOTS

Although the use of check plots involves some assumptions, it has certain advantages which safeguard an experiment that may be of long duration. Adjustment of the yields of test plots by check plots is done without introduction of *a priori* knowledge. Studies of preliminary yields have indicated that increased reliability would be obtained by adjustment based upon check plot yields in the planting under consideration. Check plots are ordinarily located at intervals which are close enough to render comparisons between the check treatment and each individual treatment rather more accurate than comparisons between any other 2 treatments. Check plots should be frequent enough to give a reliable sample of the field as a whole, and thus permit the determination of average variation, and of the effects of climatic influence and the time factor upon the variations of soil and trees.

It is thought that the possible advantages of check plots should not be overlooked in the plan of this experimental orchard. Under conditions of uniform treatment prior to the beginning of the experiment, adjustment by check plots renders significant differences of about 12 per cent in one direction between any 2 combinations of 4 plots each (table 20, values of column 6 divided by  $\sqrt{4}$ ). Differences as low as about 8.3 per cent in one direction might be significant in comparisons of any particular individual treatment of 4 plots each with the check treatment (value for *D* on page 125 divided by  $\sqrt{4}$ ). These decreases in differences necessary for significance justify the use of check plots. Provision has therefore been made for them in the future experiment.

However, the necessity of trying many treatments in the experiment requires that the number of check plots be kept as small as possible. In the above study of the effect of adjustment by means of systematically replicated checks, it was indicated that the more frequent the check plots the greater was the reduction in variation of test plots after adjustment, with the exception of the inadvisable case where the checks were located on each side of the field. The gain in precision is slight, however, as the number of check plots is increased. It seems that the release of as many plots as possible for use as test plots would outweigh the slight gain in reliability to be obtained by having checks at the more usual intervals of 3 to 5 plots. It has been decided, therefore, to use 25 check plots in the field of 199 plots. The area which they occupy is 12.6 per cent of the whole planting.

The location of these 25 check plots has been determined arbitrarily on the basis of their yields during the preliminary period of uniform culture. This has been done in such a way that they are a fair sample for the preliminary period of the yield of the field as a whole, and also of the local areas in which each plot is situated. It is planned that these plots shall be used to measure the continuity of relative differences between various sections of the field before and after the initiation of the treatments. For this reason, but chiefly to avoid confusion with the usual use of check plots, they have been called "continuity" plots.

Inspection of the data of the mean annual yield per tree of each plot for the six-year period (table 16), shows that there is frequently a tendency for groups of plots to yield approximately alike.<sup>17</sup> Consequently, these plots were grouped together as nearly as was practicable, with regard for numbers of plots in each group and for the location of the groups as a whole in relation to each other. The mean yield of each

<sup>17</sup> The more productive areas were correlated with the presence of slightly more luxuriant leaves in the early spring of 1927.

group of plots was determined. One plot in each group, the yield of which was as close as possible to the mean yield of the group, was then selected as the continuity plot. Occasional compromises were made in order to secure a more satisfactory geographical scattering of the continuity plots. The location of the plots selected is indicated in table 16.

TABLE 21

PRELIMINARY YIELD OF CONTINUITY PLOTS AND MEAN YIELD OF PLOTS IN AREA  
ADJACENT TO EACH

(Mean annual yields per tree for 1922-1927)

Continuity plots		Adjacent plots		
Location	Yield	Number of plots*	Location	Mean yield
	<i>pounds</i>			<i>pounds</i>
D8	137	6	D2 - D14	134
D24	121	6	D16 - D28	115
D38	133	8	D30 - D46	136
D48	117	3	D48 - D54	115
E4	127	6	E2 - E14	126
E20	116	4	E16 - E24	116
F36	138	5	F32 - F42	139
F52	149	4	F44 - F54	148
G24	123	7	G22 - G36	128
G44	144	8	G38 - G54	146
H16	130	6	H14 - H26	123
H36	143	6	H28 - H40	142
H48	129	5	H42 - H54	126
I4	120	6	I2 - I14	121
I28	129	14	I16 - I44	130
J8	115	10	J2 - J22	118
J34	134	10	J24 - J44	132
K6	109	5	K2 - K12	110
K20	127	7	K14 - K28	123
K44	143	7	K30 - K44	140
L10	113	10	L2 - L24	115
L30	131	8	L26 - L44	130
M4	95	7	M2 - M16	96
M24	103	7	M18 - M32	106
M40	121	5	M34 - M44	119

\* Excluding plots removed from the experiment, and continuity plots in each group.

In this table the groups of plots related to each continuity plot are also indicated. The mean annual yield per tree of each continuity plot over the six-year period is given in table 21, where it may be compared with the mean annual yield per tree of the related plots. The correlation between the mean of all related test plots and their companion continuity plots is high ( $+0.721 \pm 0.025$ ), and compares very favorably with the correlations between actual and theoretical check yields of test plots calculated from systematically distributed check plots (table 19).

Such a correlation, if maintained, should result in a decrease of error, provided adjustment based upon the use of these plots as the theoretical check yield of the group of adjacent test plots is advisable. Using the preliminary yields of blocks I to M, adjustment of the yields of test plots was found to reduce their coefficient of variation 27.4 per cent of the unadjusted variation (from 13.43 per cent to 9.75 per cent), when the assumption was made that the yield of the theoretical check for each test plot is equal to the yield of the continuity plot in the same series. When the entire 195 plots are considered, the reduction in the coefficients of variation is 30.2 per cent of the unadjusted coefficient, from 13.03 per cent to 9.10 per cent. This reduction of variation is as great as that obtained by the use of check plots systematically replicated at more frequent intervals. With the coefficient of variation of 9.10 per cent, the odds are 30 to 1 that differences of about 11.7 per cent would be significant between 2 treatments of 4 noncorrelated plots each. An advantage exists over the systematic arrangement giving similar reliability, however, since a considerable number of plots are released for experimental purposes by the arrangement used. In case the yields of the plots in each of the groups about a continuity plot fail to be correlated in the future, the plots can be handled as if they were check plots distributed at irregular intervals. This would increase the errors of comparisons between adjusted yields but slightly, presumably to a point somewhere near that which would be given by the use of check plots distributed systematically at intervals of 8 plots.

#### THE ALLOCATION OF COMBINATION PLOTS

After the selection of 25 continuity plots, there remain 170 plots satisfactory for use as test plots, since 4 plots were temporarily eliminated because of injuries to the trees. The coefficient of variation of these 170 plots is 13.03 per cent. Two plots are to be used for special purposes. The 168 remaining plots have been divided into 42 treatment plots of 4 units each. The nature of these treatments is described elsewhere.<sup>(5)</sup>

The location of the plots for each treatment has been determined arbitrarily. The following considerations have had weight in the allocation:

1. *Preliminary yield:* In order to increase the reliability of the sampling an effort was made to select plots which represent the range of variation of the orchard for each treatment. With this in view, the mean yields of the plots were arranged in ascending order, and then separated

into 4 yield groups. Four plots were selected for each treatment in such a way that the mean yield of the 4 plots for 1922-27 was practically equal to the mean yield of the field.<sup>18</sup> The plan was to select 1 plot of each quartile group for each treatment, but it was impossible to follow this procedure exactly, since compromises were occasionally made necessary because of the importance of other factors.

2. *Satisfactory geographical distribution*: This factor was considered of great importance. If the yields of a plot after the beginning of the treatments are not correlated with the yields of that plot during the preliminary period, it would be desirable to have a random distribution of the unit plots of each treatment. This could be obtained most satisfactorily on the basis of random replication.

Another very important reason for obtaining a good geographical scattering of the unit test plots of the treatments is that it helps to protect the experiment from the local effects of accidental or of climatic injuries. These factors might be very important in affecting the yields of only a part of the planting. The scattering of the plots minimizes the chance that they will seriously affect the treatment means. In case the injury is so severe as to make the elimination of the records of a part of the field advisable, it is improbable that more than 1 unit test plot of a treatment would be discarded if the plots were distributed in this way. Some results would, therefore, be obtained from the remaining plots.

3. *Ease of visual comparisons*: The desire to compare certain treatments made it advisable to locate one replication of contrasting treatments in such a way that the comparable plots may be easily observed.

4. *Ease of cultural operations*: The grouping of plots which are to be cultivated alike is important in conducting an experiment of this kind, the success of which depends upon the reliability of the field operations. In general, such plots have been grouped in pairs one above the other from east to west, and occasionally in parallel pairs from north to south. This makes it possible to hasten the seasonal operations, such as the distribution of bulky fertilizers, as well as plowing and cultivation, which differ in the various treatments and which must be performed as nearly simultaneously as practicable.

The distribution of the 42 treatments is shown in table 22.

The preliminary yields of the unit plots making up the combination, or treatment, plots are given in table 23. In this table the yields are

<sup>18</sup> This is the method used by Lehmann.<sup>(27)</sup> It was independently suggested by Mr. F. D. Richey, in correspondence concerning the present orchard, who also suggested that it might be desirable to obtain nearly equal standard deviations for the different treatments. The method employed has approximated this condition, although the distribution has additional advantages, as indicated above.

arranged in ascending order to show the distribution which was obtained for the plots of each treatment. The actual yields of each plot are given in pounds (table 23, col. 3), and also in the percentage which they constitute of the mean yield of the field as a whole for the six-year period (col. 5). It may be seen that all the combination-plot means are approximately equal (cols. 4 and 6).

TABLE 22  
DISTRIBUTION OF TREATMENTS IN THE FIELD

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
	Treatment No.									
2.....	16	23	30	26	29				10	12
4.....	C*	24	38	6	C				C	40
6.....	2	20	C	40	18				7	13
8.....	29	32	41	C	38				14	C
10.....	35	C	19	9	13				41	35
12.....	18	21	25	7	12				8	6
14.....	5	1	27	10	37	39			9	6†
16.....	4	3	31	14	42	C			11	6†
18.....	15	9	24	8	15	28			20	1
20.....	19	8	C	33	36	16			C	31
22.....	17	6	17	11	19	22	27		5	2
24.....	C	—	32	39	24	21	C		42	C
26.....	13	10	36	28	25	23	38			4
28.....	12	7	18	16	C	35	39			3
30.....	14	C	15	22	30	26	36			30
32.....	33	11	5	20	27	6	24	19		21
34.....	31	—	42	C	17	7	32	33		37
36.....	36	38	29	34	31	C	40	C		34
38.....	22	28	4	1	32	37	1	2		C
40.....	C	40	3	2	21	10	3	20		28
42.....	30	26	16	23	9	11	4	5		23
44.....	39	37	C	35	41	—	C	29		24
46.....						8	25	17		15
48.....						C	12	22		C
50.....						14	13	18		26
52.....						34	41	C		27
54.....						33	42	—		25

\* C represents continuity plot.

† Demonstration plots.

#### FUTURE COMPARISONS

The statistical analysis of the reliability of the differences which may be obtained between treatments in the future trials can be made by several methods. However, the feasibility of comparisons between treatments based on differences between plots which were correlated in yield during the preliminary period of testing will be an object of future study. For the purposes of such comparisons, differences would be



TABLE 23  
MEAN ANNUAL YIELDS PER TREE OF UNIT AND COMBINATION PLOTS, 1922-1927

Treatment No.	Plot numbers in order of increasing yields				Mean yield of respective unit plots, pounds*				Mean yield of combination plots, pounds		Mean yield of respective unit plots in per cent of mean yield of field		Mean yield of the combination plots in per cent	
	2				3				4		5			6
1.....	D18	J38	L14	G38	105	124	126	148	125.75	84	99	100	118	100.25
2.....	M6	D22	F38	J40	111	124	129	139	125.75	88	99	103	111	100.25
3.....	D28	L16	G40	K40	116	116	133	135	125.00	92	92	106	107	99.75
4.....	M16	K38	D26	G42	100	119	130	155	126.00	80	95	103	123	100.25
5.....	M14	E22	K32	F42	93	122	135	153	125.75	74	97	107	122	100.00
6.....	J4	H32	D12	L22	104	129	133	138	126.00	83	103	106	110	100.50
7.....	E6	L28	J12	H34	116	119	128	139	125.50	92	95	102	111	100.00
8.....	L20	J18	H46	E12	116	119	126	140	125.25	92	95	100	111	99.50
9.....	J10	L18	E14	I42	110	115	133	144	125.50	88	91	106	115	100.00
10.....	E2	L26	J14	H40	100	121	128	150	124.75	80	96	102	119	99.75
11.....	E16	L32	J22	H42	100	127	137	142	126.50	80	101	109	113	100.75
12.....	I12	M28	D2	G48	113	119	121	150	125.75	90	95	96	119	100.00
13.....	M26	I10	D6	G50	97	122	141	142	125.50	77	97	112	113	99.75
14.....	M30	J16	H50	E8	108	119	134	139	125.00	86	95	107	111	99.75
15.....	M18	I18	D46	K30	93	120	146	148	126.75	74	95	116	118	100.75
16.....	M2	H20	J28	K42	87	131	132	157	126.75	69	104	105	125	100.75
17.....	M22	I34	K22	F46	98	121	132	155	126.50	78	96	105	123	100.50
18.....	M12	K28	F50	I6	102	129	131	140	125.50	81	103	104	111	99.75
19.....	M20	K10	I22	F32	98	116	138	152	126.00	78	92	110	121	100.25
20.....	L6	E18	F40	J32	110	124	131	134	124.75	88	99	104	107	99.50
21.....	L12	H24	F48	I40	109	114	140	142	126.25	87	91	111	113	100.50

\* These 4 subcolumns represent the 4 yield groups selected by the method described on pp. 137-138. Mean annual yield of all plots = 125.7 pounds.

TABLE 23—(Concluded)

Treatment No.	Plot numbers in order of increasing yields	Mean yield of respective unit plots, pounds*			Mean yield of combination plots, pounds	Mean yield of respective unit plots in per cent of mean yield of field		Mean yield of the combination plots in per cent
		2	3	4		5	6	
22.....	H22 M38 J30 D32	116 120 130 139	126.25	82 95 103 111	100.25			
23.....	L2 H26 D42 J42	103 115 138 146	125.50	82 91 110 116	99.75			
24.....	K18 I24 G32 D44	112 115 133 144	126.00	89 91 106 115	100.25			
25.....	D64 I26 K12 G46	108 117 122 155	125.50	86 93 97 123	99.75			
26.....	J2 D60 L42 H30	103 115 128 153	124.75	82 91 102 122	99.25			
27.....	G22 D52 I32 K14	120 122 126 137	126.25	95 97 100 109	100.25			
28.....	J26 D40 H18 L38	110 122 132 137	125.25	88 97 105 109	99.75			
29.....	M8 I2 K36 F44	79 113 146 165	125.75	63 90 116 131	100.00			
30.....	K2 M42 D30 I30	96 114 136 152	124.50	76 91 108 121	99.00			
31.....	D20 M34 K16 I36	105 119 129 150	125.75	84 95 103 119	100.25			
32.....	L8 K24 G34 I38	112 121 135 136	126.00	89 96 107 108	100.00			
33.....	H54 M32 F34 J20	114 127 128 135	126.00	91 101 102 107	100.25			
34.....	L4 H52 D36 J36	106 114 136 147	125.75	84 91 108 117	100.00			
35.....	M10 D10 J44 H28	98 128 135 138	124.75	78 102 107 110	99.25			
36.....	K26 I20 M36 G30	104 125 135 140	126.00	83 99 107 111	100.00			
37.....	I14 L44 D34 H38	108 126 128 142	126.00	86 100 102 113	100.25			
38.....	G26 K4 I8 L36	103 119 129 147	124.50	82 95 103 117	99.25			
39.....	M44 J24 H14 G28	107 119 132 141	124.75	85 95 105 112	99.25			
40.....	J6 G36 L40 D4	99 127 138 140	126.00	79 101 110 111	100.25			
41.....	K8 I44 E10 G52	99 126 130 150	126.25	79 100 103 119	100.25			
42.....	I16 E24 G54 K34	113 118 136 140	126.75	90 94 108 111	100.75			

\* These 4 subcolumns represent the 4 yield groups selected by the method described on pp. 137-138. Mean annual yield of all plots = 125.7 pounds.

obtained between pairs of the unit plots, the preliminary yields of each pair being in the same yield group as indicated by their position in the same vertical subcolumn of table 23, column 3.

The magnitude of the differences between treatment means which would have been necessary for any given level of significance if the orchard, as finally laid out, had been under differential treatment from 1922 through 1927, can be determined from the available data. According to Student's<sup>(59)</sup> method for determining the average variance of a difference between the mean yields of the treatments as allocated above,  $\sigma_d^2 = 3.9$  pounds. The probable error of the mean difference is, therefore, 1.33 pounds or 1.06 per cent of the mean yield per tree of all plots. If odds of 30 to 1 are desired for significance, this last figure is multiplied by 2.7 to obtain the average percentage difference between treatment means necessary; this is only 2.86 per cent of the mean annual yield per tree of all plots.

The correlation between plots in the same yield group cannot be expected to remain as high in the future years of the experiment as during the period of preliminary observation. In this event larger differences than those just noted will be necessary for significance. It seems probable, however, that a significant correlation will exist for many years, if not for the duration of the experiment, in which event it will have the effect of reducing the error of the experiment. If this correlation becomes small it is possible that methods of interpretation based upon adjustment of yields to check plots, or upon other methods, may lead to more accurate conclusions.

#### SOME RELATIONS OF THE VARIATION OF MEASUREMENTS OF TREE SIZE TO THE PLAN OF THE EXPERIMENT

Up to the present point the discussion of the plan of the experiment has been concerned chiefly with data of yields. Although yield data may be considered the most important criterion of the effects of the cultural treatments which are to be experimented with, they should be supplemented with other measures, particularly of the growth of the trees. According to the plans adopted, the area of cross section of the smallest point on the trunk and the volume of the top of the tree will be used as indexes of this response. Trunk measurements have been made annually since 1918, while careful top-volume measurements have been made since 1922.

Although it appears unnecessary for the purpose of the present paper to enter into a detailed analysis of the variation of the size of

TABLE 24  
STATISTICAL CONSTANTS FOR MEASUREMENTS OF SIZE OF TREES IN NOVEMBER  
OF VARIOUS YEARS

Year, fall of	Top volume of individual trees			Coefficient of variation	Mean*	Standard deviation	Top volume per tree of plots			Coefficient of variation
	<i>cubic feet</i>	<i>cubic feet</i>	<i>per cent</i>				<i>cubic feet</i>	<i>cubic feet</i>	<i>per cent</i>	
1922	374.9±1.16	67.0±0.82	17.87±0.226		376.3±1.93		39.9±1.36		10.61±0.366	
1923	487.7±1.53	88.5±1.08	18.15±0.231		487.9±2.36		48.9±1.67		10.02±0.346	
1924	654.0±2.01	116.0±1.42	17.74±0.224		653.2±3.18		65.9±2.25		10.09±0.348	
1925	749.8±2.27	131.0±1.60	17.47±0.220		748.6±3.51		72.6±2.48		9.70±0.334	
1926	848.2±2.58	149.0±1.83	17.57±0.222		849.1±3.77		78.0±2.66		9.19±0.316	
	Area of trunk cross section of individual trees						Area of trunk cross section† per tree of plots			
	<i>square centimeters</i>	<i>square centimeters</i>	<i>per cent</i>				<i>square centimeters</i>	<i>square centimeters</i>	<i>per cent</i>	
1921	57.32±0.156	9.00±0.110	15.70±0.197		56.89±0.214		4.44±0.150		9.80±0.267	
1922	74.99±0.219	12.65±0.155	16.87±0.212		74.85±0.285		5.90±0.201		7.88±0.270	
1923	90.86±0.226	13.05±0.160	14.36±0.179		90.65±0.336		6.95±0.237		7.67±0.263	
1924	102.80±0.255	14.75±0.181	14.35±0.179		102.30±0.382		7.90±0.270		7.72±0.265	
1925	117.41±0.295	17.00±0.208	14.48±0.181		116.90±0.442		9.15±0.312		7.93±0.268	
1926	127.22±0.314	18.10±0.222	14.23±0.178		126.90±0.447		9.25±0.316		7.29±0.250	

\* From grouped data.

† Area of cross section at a constant point on the trunk.

the trees as indicated by trunk size and volume of top, certain statistics will be of value, especially in showing some relations of these criteria to the plan of the experiment as laid out.

In table 24 are recorded the means, standard deviations, and coefficients of variation of the size measurements of trees and also the mean

TABLE 25  
MEAN TOP VOLUME IN CUBIC FEET PER TREE FOR EACH PLOT, NOVEMBER, 1926

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2	733	758	757	780	927				874	868
4	725	769	868	703	858				954	921
6	845	761	815	723	947				761	829
8	776	750	784	817	934				909	1,014
10	755	777	800	869	1,014				936	936
12	735	825	818	801	926				848	814
14	853	887	876	791	921	1,115			881	783
16	812	831	888	804	855	992			596	710
18	787	827	825	803	921	917			860	895
20	699	821	935	893	945	1,001			836	811
22	803	780	891	969	995	983	901		802	871
24	764	*	857	944	858	1,005	898		761	799
26	759	799	778	819	857	979	829			889
28	862	823	863	840	863	965	973			839
30	756	754	847	887	854	930	866			877
32	799	791	799	904	845	797	918	1,071		784
34	738	*	845	780	942	866	919	916		732
36	750	790	843	848	947	901	975	976		813
38	727	801	817	771	806	807	927	747		809
40	791	819	826	867	848	878	854	865		830
42	696	730	885	822	894	882	789	905		811
44	710	813	832	850	796	*	873	896		849
46						815	885	963		944
48						843	849	901		750
50						840	840	895		924
52						829	920	1,006		847
54						855	959	*		823

\* Plots omitted because of injury to trees.

size per tree of all plots, as determined in the fall of the years 1922 to 1926 inclusive. The abnormal trees eliminated from the records of yields are not included in the data. The coefficients of variation emphasize the relative uniformity of the size of the trees. However, large individual fluctuations are easily possible with the variation recorded. This is verified by a study of the mean size per tree of plots in the various years, examples of which are given for the fall measurements of 1926 in tables 25 and 26.

It may also be observed upon reference to tables 2, 11, and 24, that the variability in the size of tree at any one time of measurement is

less than that of the yields in the same crop year. As in the case of yields, the means of the measurements of the trees in each plot show less variation than do the measurements of individual trees. The reduction in the coefficients is not, however, as great as that which would have been expected by the random combination of the same number of trees.

TABLE 26  
MEAN AREA OF TRUNK CROSS SECTION IN SQUARE CENTIMETERS PER TREE  
FOR EACH PLOT, NOVEMBER, 1926

Plot	Blocks									
	M	L	K	J	I	H	G	F	E	D
2.....	111	109	104	117	129				132	134
4.....	111	114	112	105	129				134	142
6.....	118	114	113	107	128				121	127
8.....	119	113	109	129	133				130	139
10.....	115	120	127	123	141				135	136
12.....	113	125	122	117	130				135	129
14.....	126	121	137	120	146	153			132	128
16.....	122	122	131	120	135	148			103	114
18.....	122	117	120	127	149	136			133	131
20.....	115	115	126	127	143	142			128	126
22.....	129	122	133	127	134	140	140		129	127
24.....	132	*	127	124	129	137	133		118	125
26.....	132	126	117	131	137	128	129			128
28.....	127	122	141	127	123	127	134			128
30.....	128	121	133	139	132	127	130			137
32.....	120	119	128	126	138	122	129	144		132
34.....	114	*	135	119	139	132	135	124		125
36.....	122	119	133	123	133	136	137	132		126
38.....	107	117	114	123	122	128	136	125		125
40.....	128	118	134	124	118	123	126	123		130
42.....	118	110	120	118	136	123	131	132		128
44.....	112	121	132	130	113	*	127	131		133
46.....						118	134	147		139
48.....						127	132	125		126
50.....						131	128	128		137
52.....						129	134	143		138
54.....						135	142	*		131

\* Plots omitted because of injury to trees.

It is apparent that the sizes of trees in the same plot are correlated, and that systematic influences affect their growth in various parts of the orchard.

A striking point in the data of table 24 is that during the years 1922 to 1926 inclusive, the variation of the size measures of trees and of plots fluctuates very little in the various years. This suggests that there is a high positive correlation in the sizes of trees in the various years. In fact, the coefficient of correlation between the mean top volume per tree of each plot in 1922 with that in 1926 has been found to be + 0.693

$\pm 0.025$ . The coefficient of correlation for the mean area of cross section of the trunk of the trees of each plot for the same dates is  $+0.889 \pm 0.010$ . These high positive correlations suggest that the relative size of trees in the different plots may tend to be more or less constant for a considerable period of time. This is in agreement with the observation upon orange trees of Webber,<sup>(64, 65, 66, 67)</sup> and Webber and Barrett,<sup>(68)</sup> and also with the conclusions of Sax.<sup>(45, 46)</sup>

TABLE 27

CORRELATION COEFFICIENTS OF YIELD OF TREES AND PLOTS WITH AREA OF TRUNK CROSS SECTION AND TOP VOLUME IN THE SAME CROP YEAR\*

Year, spring of	Coefficients of correlation between yield and size	
	On the basis of individual trees	On the basis of plot means
	Size measured by area of trunk cross section	
1923.....	$+0.109 \pm 0.017$	$+0.220 \pm 0.046$
1924.....	$+0.233 \pm 0.016$	$+0.590 \pm 0.031$
1925.....	$+0.247 \pm 0.016$	$+0.307 \pm 0.044$
1926.....	$+0.278 \pm 0.016$	$+0.331 \pm 0.043$
1927.....	$+0.322 \pm 0.016$	$+0.342 \pm 0.043$
	Size measured by top volume	
1923.....	$+0.248 \pm 0.016$	$+0.335 \pm 0.043$
1924.....	$+0.478 \pm 0.013$	$+0.625 \pm 0.029$
1925.....	$+0.331 \pm 0.015$	$+0.453 \pm 0.038$
1926.....	$+0.286 \pm 0.016$	$+0.240 \pm 0.046$
1927.....	$+0.297 \pm 0.016$	$+0.341 \pm 0.043$

\* Total populations: 1,513 to 1,519 trees and 195 plots.

The relations of the sizes of the trees to their yields are indicated in table 27. In this table the coefficient of correlation between the cross-section area of the trunk, and also between the volume of individual trees, with the yields of the same trees in the same crop year, are presented. Similar coefficients are given for the correlation between the mean size and the mean yield per tree of plots for those years. The correlations are all positive and significant. This information indicates that in the trees of this planting there has been a tendency for the larger trees to be the higher producers.

The question naturally arises as to whether size of top or size of trunk is a better index of growth. The two measures are highly correlated in the records available at the present time. Thus in 1922 the coefficient of correlation between cross section and top volume, on a

plot basis, was  $+0.701 \pm 0.025$ . In 1926 the same calculation rendered a coefficient of  $+0.717 \pm 0.023$ . On a tree basis the correlation between the two measures in 1926 was  $+0.659 \pm 0.010$ . However, the coefficients given in table 27 indicate that in most years the correlation between yield and top volume was only slightly higher than that between

TABLE 28

MEAN AREA OF TRUNK CROSS SECTION AND MEAN TOP VOLUME PER TREE OF CONTINUITY PLOTS AND MEAN OF PLOTS CONTIGUOUS TO EACH\*

Location of continuity plots	Location of adjacent plots	Mean area of trunk cross section, in square centimeters per tree		Mean top volume, in cubic feet per tree	
		Continuity plots	Mean of adjacent plots	Continuity plots	Mean of adjacent plots
D8	D2 - D14	139	133	1,014	859
D24	D16 - D28	125	126	799	836
D38	D30 - D46	125	131	809	830
D48	D48 - D54	126	135	750	865
F4	E2 - E14	134	131	954	868
F20	E16 - E24	128	121	836	755
F36	F32 - F42	132	130	976	901
F52	F44 - F54	143	133	1,006	914
G24	G22 - G36	133	133	898	912
G44	G38 - G54	127	133	873	878
H16	H14 - H26	148	139	992	833
H36	H28 - H40	136	127	901	874
H48	H42 - H54	127	127	843	844
I4	I2 - I14	129	135	858	945
I28	I16 - I44	123	133	863	883
J8	J2 - J22	129	119	817	814
J34	J24 - J44	119	127	780	855
K6	K2 - K12	113	115	815	805
K20	K14 - K28	126	129	935	854
K44	K30 - K44	132	128	832	837
L10	L2 - L24	120	117	777	801
L36	L26 - L44	121	119	754	796
M4	M2 - M16	111	118	725	787
M24	M18 - M32	132	125	764	781
M40	M34 - M44	128	115	791	724

\* Measured in November, 1926.

yield and area of cross section of trunk. It should be emphasized that the trees were growing fairly vigorously during this period. (See table 24 for mean size in the different years.) It seems possible that if accidental injury or the effects of treatments reduce the volume of the top greatly, top volume might become the better criterion.

At the conclusion of a preliminary period of testing, it is possible that size might be a better criterion of future productivity than yield and that the experiment might be planned more satisfactorily on that



TABLE 29  
MEAN TOP VOLUME AND MEAN AREA OF TRUNK CROSS SECTION OF UNIT AND  
COMBINATION PLOTS, NOVEMBER, 1926

Treatment No.	Mean top volume, in cubic feet per tree					Mean area of trunk cross section in square centimeters per tree				
	Of respective plots*				Mean	Of respective plots*				Mean
1.....	895	771	887	927	870	131	123	121	136	128
2.....	845	871	747	867	833	118	127	125	124	124
3.....	839	831	854	826	838	128	122	126	134	128
4.....	812	817	889	789	827	122	114	128	131	124
5.....	853	802	799	905	840	126	129	128	132	129
6.....	708	797	814	780	774	105	122	129	122	120
7.....	761	823	801	866	813	121	122	117	132	123
8.....	821	803	815	848	822	115	127	118	135	124
9.....	869	827	881	894	868	123	117	132	136	127
10.....	874	799	791	878	836	132	126	120	123	125
11.....	596	791	969	882	810	103	119	127	123	118
12.....	926	862	868	849	876	130	127	134	132	131
13.....	759	1,014	829	840	861	132	141	127	128	132
14.....	756	804	840	909	827	128	120	131	130	127
15.....	787	921	944	847	875	122	149	139	133	136
16.....	725	1,001	840	885	863	111	142	127	120	125
17.....	803	942	891	963	900	129	139	133	147	137
18.....	735	863	895	947	860	113	141	128	128	128
19.....	699	800	995	1,071	891	115	127	134	144	130
20.....	761	860	865	904	848	114	133	123	126	124
21.....	825	1,005	901	848	895	125	137	125	118	126
22.....	983	727	887	784	845	140	107	139	132	130
23.....	758	979	811	822	843	109	128	128	118	121
24.....	825	858	918	849	863	120	129	129	133	128
25.....	823	857	818	885	846	131	137	122	134	131
26.....	780	924	730	930	841	117	137	110	127	123
27.....	901	847	845	876	867	140	138	138	137	138
28.....	819	830	917	801	842	131	130	136	117	129
29.....	776	927	843	896	861	119	129	133	131	128
30.....	757	696	877	854	796	104	118	137	132	123
31.....	811	738	888	947	846	126	114	131	133	126
32.....	750	857	806	919	833	113	127	122	135	124
33.....	855	799	916	893	866	135	120	124	127	127
34.....	769	829	813	848	815	114	129	126	123	123
35.....	755	936	850	965	877	115	136	130	127	127
36.....	778	945	750	866	835	117	143	122	130	128
37.....	921	813	732	807	818	146	121	125	128	130
38.....	829	868	934	790	855	129	112	133	119	123
39.....	710	944	1,115	973	936	112	124	153	134	131
40.....	723	975	819	921	860	107	137	118	142	126
41.....	784	796	936	920	859	109	113	135	134	123
42.....	855	761	959	845	855	135	118	142	135	133
Mean of all plots.....					849					127

\* The order of the plots is that of increasing yields, as given in table 23.

basis. In the present experiment with Washington Navel oranges, it is anticipated that the growth responses of the trees to the different treatments can be interpreted with increased satisfaction because the preliminary records of size are available. It also seems possible that if size at the beginning of the differential treatments should be a better index of future yields than past yields, then size may be taken into account in the interpretation of yield data.

The relation of the mean size per tree of the plots to the future experiment, as planned, is indicated in tables 28 and 29. Table 28 shows the mean area of cross section of the trunks of the trees and their mean top volume in the continuity plots, and in plots contiguous to them, in a manner similar to the yield data of table 21. From the data of these tables it may be seen that there was considerable difference in the size of the trees in the different areas of the orchard at the start of the experiment, and that the mean size of trees in the plots chosen for the continuity treatment is on the whole correlated with the mean size of trees in contiguous plots.

In table 29 the size measurements per tree for 1926 are given for the plots of each treatment, and the means of the values for the 4 plots are also recorded. These means indicate that the plan of the experiment on the basis of yield during a six-year period has resulted in providing each treatment with a group of plots the mean size per tree of which was, generally, very uniform at the time the experiments were begun. However, some noticeable exceptions between treatment means do exist. If it should be indicated by future records that these differences are important in influencing yields, it may be possible that adjustment of yields by them might be advantageous.

## SUMMARY

The fluctuations of yield of plants in experimental fields that are independent of the factors under trial, are of such importance that they must be taken into account in the plan of the experimental field and in the interpretation of the results obtained. Such difficulties are especially great in experimentation with trees because of the area of land involved and the long life of the plants. These considerations greatly increase the difficulty of securing a representative sample of the planting for each treatment.

Various methods have been proposed for the planning of experiments and interpretation of experimental results obtained under field conditions. Studies of uniformly treated fields and orchards have sug-

gested that the application of certain principles may depend on the individual characteristics of the field for their effect. The study of the characteristics of the individual orchard while under conditions of uniform treatment before the beginning of the experiment proper has been emphasized as having a bearing upon the plan of the future trials. The results of such a study are herein recorded.

The material consists of 199 plots of Washington Navel orange trees planted on land which had been originally uniformly cropped to grains under dry-farming conditions. The plots are of 8 trees each, arranged in a single row. Plot rows are separated by guard rows of trees of other citrus fruits. The rootstocks, buds, and nursery trees were carefully selected in an attempt to secure uniformity. The nursery trees were well mixed and planted at random. The irrigation system was arranged so that each plot could be separately irrigated in accordance with soil-moisture conditions. The orchard was maintained under as nearly uniform conditions as practicable until 7 crops (1921 to 1927 inclusive) had been harvested. At the end of that time the differential treatments were put into effect.

It was observed that the distribution of yields of trees approached that of the normal frequency curves in six of the seven years for which studies were made. In the first year of bearing (1921), the distribution was not normal. The mean yield per tree of plots in all years was of practically normal distribution. The use of statistical formulas based upon an assumption of normality in treatment of most of the data is believed warranted.

Studies of variability of the yields of individual trees indicated a very high coefficient of variation for 1921. Coefficients for the six remaining years averaged 25.4 per cent, a relatively low figure, especially in consideration of the plan of planting. When the yields per tree of the individual plots were considered for the various years, the variation was reduced materially, but not to the extent which would be anticipated on the basis of random sampling. This phenomenon was due to a positive correlation of considerable magnitude which was found to exist between yields of trees in the same plots. Emphasis is placed upon systematic variations due to soil influences.

It was observed that the yields of all years except 1921 tended to have about the same degree of gross variation. Consideration of the mean annual yield per tree of plots in the various years, expressed in percentage of the mean annual yield of all trees, showed that there was a tendency for individual plots to yield about the same relative amount in all years except 1921. This tendency was measured by the use of

interannual correlations of yields of individual trees and of plots. In this study, the yields of 1922 and of 1923 were not as highly or as consistently correlated with yields of later years, as the yields of these later years were correlated with each other. The coefficients of variation of yields of 1922 and 1923 were also higher.

It was apparent that the use of yield data for the first year of production would not have led to results which would have been duplicated in succeeding years if the orchard had been under differential treatment at the time. The yields of 1921 were, therefore, not used in studies of various possible plans for the future experiment which are reported. The yields for the six-year period, 1922 to 1927 inclusive, are, however, rather consistent, and are used as an index of the productivity of each plot during the preliminary period. The variation of the six-year mean yield was found to be less than that of the yields of individual years, but not so low as that which would be expected on the assumption of random sampling. This effect is due to the positive interannual correlation existing between yields of the same plots in the different years.

The calculations made upon data obtained during the preliminary period of observation, during which the orchard may be regarded as a uniformity, or blank, experiment, seem to justify the expectation that an experimental plan which would have been most reliable in the preliminary period would also be most efficient in the future. The records of mean yields for six years were, therefore, subjected to a study to determine the effect of various plans upon variability of test plots, and the magnitude of the differences between the mean yields of combination, or treatment, plots which would be necessary to insure significance.

It was shown that the use of single plots for each treatment would be unsatisfactory, owing to the great differences which occur normally between them. Increase of the area devoted to each treatment by combining contiguous plots decreased natural variations between treatments, but this decrease was not rapid because of correlation between yields of adjacent plots due to systematic influences of soil fertility. The combination of systematically replicated plots for each treatment, however, reduced the variation of treatments approximately according to the expectations of random sampling. The size of the combination plots necessary to insure a reasonable degree of significance under these conditions was found to be larger than desirable.

Certain aspects are presented of the theory of the use of check plots in attempts to reduce systematic variations due to fluctuations in the fertility of the soil. Attempts have been made to determine the effects of adjustment of test-plot yield by means of systematically replicated

check plots. Checks were located at various intervals, and several of the more common methods of calculating the theoretical check yields of the intervening test plots were used. The greatest reduction in variation of adjusted yields was found when check plots were located at frequent intervals and an interpolation, or "grading," formula was used for the calculation of theoretical check yields of test plots. Differences between treatment means necessary for a moderate degree of significance were also calculated, and showed corresponding decreases. It is pointed out, however, that the use of check plots requires a large area of land which might be used for increased replications of the treatments. By assuming that a constant number of hypothetical treatments were to be tried upon the area studied, the number of test plots devoted to each varying according to the number of checks employed, it was found that the most favorable gain in reliability obtained by adjustment to checks was slightly greater than that obtained by increased replication of test plots for each treatment which was made possible by the elimination of the check plots.

It was observed that the use of differences between yields of test plots, adjusted by means of check plots, and the yields of the check plots themselves reduced systematic variation in yield due to soil fluctuations. Rather accurate conclusions can be drawn by this method as to the significance of small differences in yield between any 1 treatment and the check plots. This advantage, however, vanishes when it is desirable to compare 2 treatments by means of the difference between their adjusted yields and the check yield.

The use of methods of differences between test plots was found by means of Student's<sup>(59)</sup> formula to give about the same reliability, with many treatments and a small number of systematically distributed replicates for each treatment, as that obtained in direct comparisons between the means of the treatments. This indicates that little or no correlation exists between the "paired" plots of a replication series under such conditions, and that the last term in the formula for the variance of a difference,  $\sigma_1^2 + \sigma_2^2 - 2r_{1.2}\sigma_1\sigma_2$ , is practically equal to zero.

The adjustment of yields of test plots by means of other contiguous test plots is discussed. It is believed that the value of the use of such methods can be obtained after the differential trials are in effect.

The use of yields of test plots, obtained under conditions of uniform culture, for the adjustment of yields of the same plots after the different treatments have gone into effect is discussed. There are certain aspects of experimental work with trees which indicate that interannual correlations of yields with orchard material may be more consistently positive

than with annual plants. The calculations made upon the data of this orchard suggest that they may be important. It is believed that methods may be used to take advantage of whatever correlations may be found in the future between yields prior to and during the actual period of testing the various treatments. It is probable that pairing plot yields of individual treatments may be carried out in the future trial upon the basis of their correlated yields during the preliminary period of testing.

As a result of these studies a plan for the experimental orchard under consideration has been adapted to the practical ends desired. Four plots are used for each treatment and are chosen on the basis of preliminary yield. Check plots designated as continuity plots, are provided as a precautionary measure, and to obtain certain information as to orchard experimental technique. The check plots are also arranged on the basis of preliminary yields in such a way that they are a fair sample of the area contiguous to them at the time of starting the differential treatments, with the idea of judging the relative productivity of that area. Adjustment of yields of test plots by the use of the continuity plots, under the conditions existing during the preliminary testing, gave an increase in reliability comparable to that given by the use of systematically replicated check plots at more frequent intervals.

The 4 test plots allocated to each treatment were chosen on the basis of their mean annual yield per tree for the six-year period in such a way that the mean yields of the sets of 4 plots for this period were approximately equal. Wherever feasible, 1 plot was chosen from each quartile group of the frequency distribution, forming 4 replication series. The importance of a good geographical scattering of the replicates and of certain features which are important from a cultural point of view was also recognized in selecting the replicates for each treatment. With the high correlation existing between yields of plots in the same yield group during the preliminary period, the use of Student's<sup>(69)</sup> method for the determination of the average variance of a difference between treatments indicated that relatively small differences would be significant with a considerable degree of reliability. The reliability of small differences in the future as determined by this method depends upon the correlation between yields of plots in the 4 yield groups. Should this correlation vanish entirely, a possibility which does not appear imminent, the more common methods of interpretation may be used, such as adjustment by means of check plots or contiguous test plots, or by comparisons between unadjusted yields.

Some relations of the plan of the experiment, as developed on the basis of past yields, to the variability of measures of tree size are

presented. Yield was somewhat more variable than the volume of the top of the tree, or the area of the cross section of the trunk. Interannual correlations between the size measurements were high. These correlations emphasize the value of a knowledge of the size of trees before the beginning of an experiment as an aid in the interpretation of the effects of the treatments. Since the size of these trees at any one period was correlated with their yield in the same crop year, a knowledge of the size at the beginning of an experiment might logically be used as a basis of pairing trees or plots for comparison of yields by methods of differences. The tree-size relations of the plots of the experimental orchard, as planned on the basis of yields, are presented. The data indicate that, on the whole, the mean size of the trees devoted to each treatment approaches the mean of the orchard. Occasional differences of considerable dimensions do exist, however. It is possible that future records may show that some recognition of these differences should be made and that some adjustment may be desirable.

## ACKNOWLEDGMENTS

The first author desires to express his gratitude to administrative officers of the University of California for the opportunity afforded him of pursuing this study at the Citrus Experiment Station, and for the privilege of continuing the study at Cornell University during the period of a transfer of residence and a leave of absence which were granted him for this purpose. The major portion of this paper was presented by him in February, 1929, in the Graduate School of Cornell University, as a thesis in partial fulfillment of the requirements of the degree of Doctor of Philosophy. At the latter institution the advice and criticisms of Professors H. H. Love, C. H. Myers, and A. J. Heinicke were very helpful and are highly appreciated.

The authors also desire to thank all those with whom this problem has been discussed. The results of such discussion have helped to clarify their views and to emphasize various methods of treatment of the material. Some of the suggestions made receive proper acknowledgment in the text. At the University of California the authors desire to thank Professors G. F. McEwen, H. B. Frost, J. G. Surr, C. M. Titus, and H. J. Webber, particularly. At other institutions, contact with the aforementioned members of the staff of Cornell University, and also with R. D. Anthony (Pennsylvania State College), R. A. Fisher (Rothamsted Experiment Station, England), H. K. Hayes (University of Minnesota), F. D. Richey (United States Department of Agriculture), K. Sax (now at Harvard University), and L. J. Stadler (University of Missouri) has been especially helpful. None of the above-mentioned men, however, should be held responsible for errors of mathematical reasoning or application which may appear in the paper.



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## APPENDIX

TABLE OF ODDS\*

Difference from mean in terms of probable error	Difference between two results in terms of probable error	Odds against such a difference occurring under uniform conditions
With difference in either direction		
1.00	1.41	1 to 1
1.25	1.76	3 to 2
1.44	2.03	2 to 1
1.71	2.41	3 to 1
1.90	2.68	4 to 1
2.00	2.83	9 to 2
2.05	2.87	5 to 1
2.50	3.53	10 to 1
2.93	4.13	20 to 1
3.00	4.24	22 to 1
3.20	4.51	30 to 1
4.00	5.66	140 to 1
4.90	6.93	1,000 to 1
5.00	7.07	1,350 to 1
With difference in one direction only		
1.00	1.41	3 to 1
1.25	1.76	4 to 1
1.44	2.03	5 to 1
1.58	2.23	6 to 1
1.71	2.41	7 to 1
1.81	2.55	8 to 1
1.90	2.68	9 to 1
2.00	2.83	10 to 1
2.48	3.50	20 to 1
2.70	3.81	30 to 1
2.89	4.07	40 to 1
3.00	4.24	44 to 1
3.03	4.28	50 to 1
3.44	4.85	100 to 1
4.00	5.66	290 to 1
5.00	7.07	2,700 to 1

\* From Wood, T. B. The interpretation of experimental results. Jour. Bd. Agr. [London] Sup. 17:15-32. 1911.



# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

NOVEMBER, 1932

No. 3

## TRANSMISSION OF CARROT, PARSLEY, AND PARSNIP YELLOWS BY CICADULA DIVISA<sup>1</sup>

HENRY H. P. SEVERIN<sup>2</sup>

(Contribution from the Division of Entomology and Parasitology, California Agricultural Experiment Station, University of California, cooperating with the United States Department of Agriculture, Bureau of Entomology.)

### INTRODUCTION

A number of plant pathologists have called attention to a disease of carrots having most of the characteristic symptoms of yellows, but whether it was caused by the aster-yellows virus remained to be determined.

In New York, Whetzel<sup>(8)</sup> reported a yellows disease of carrots ranging from a trace to 25 per cent infection in the Williamson area. Folsom<sup>(1)</sup> found apparently the same carrot disease as described by Whetzel, at Orono, Maine, and on the experimental farm in the southwestern part of the state. Zundel<sup>(9)</sup> reported observations of yellows believed to be caused by the aster-yellows virus in carrots in Cumberland County, Pennsylvania. Vaughan and Foster<sup>(7)</sup> found a disease of carrots in Wisconsin resembling aster yellows and assumed that it may be due to the same virus. The diseased carrots were growing adjacent to an experimental aster-yellows plot. Newhall<sup>(3)</sup> reported a disease of carrots thought to be due to the aster-yellows virus in Wayne and Oswego counties, New York.

Severin<sup>(6)</sup> published a summary of the transmission of yellows from naturally and experimentally infected carrots and parsley to asters and

<sup>1</sup> Received for publication May 25, 1932.

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celery, and from the infected asters and celery back to carrots and parsley. Kunkel,<sup>(2)</sup> however, questions whether carrot yellows of California is identical with aster yellows of the East, since carrot yellows is readily transmitted to celery (*Apium graveolens* var. *dulce*) and to *Zinnia elegans*, plants that are highly resistant if not immune to aster yellows.

During the spring and late summer of 1929 a survey was made of the yellows disease of plants grown on the ranch of the Morse Seed Company located in the Salinas Valley, California. It was observed during early June that the inner leaves of many varieties of carrots were yellow, but the plants were small and the symptoms were just beginning to develop. During the late summer the white varieties of carrots and parsley showed some of the characteristic symptoms of yellows but, unfortunately, the orange varieties of carrots had been pulled.

During the summer of 1931 another trip was taken to the ranch of the Morse Seed Company and both white and orange varieties of carrots showed typical symptoms of yellows. Parsnips also showed symptoms of yellows. Carrot yellows was also found in the truck-crop gardens between Davis and Sacramento.

An investigation was undertaken to determine whether the disease of carrots, parsley, and parsnips was caused by the virus of aster and celery yellows. Successive inoculations were attempted from asters and celery naturally and experimentally infected with yellows to healthy carrots, parsley, and parsnips by means of *Cicadula divisa* Uhl. The transfer of yellows from experimentally infected carrots, parsley, and parsnips back to healthy asters and celery was attempted with previously non-infective leafhoppers. Cross inoculations were tested from infected carrots to healthy ones, from parsley to parsley, carrots to parsley, and parsley to carrots. The longevity of the leafhopper and the life cycle on carrots, parsley, and parsnips was also determined.

In this investigation, the virus of carrot yellows has been compared only with that of the aster yellows present in this state; the question of whether the carrot-yellows virus is identical with the aster yellows of the eastern and middle western states is under investigation at present.

## CARROT YELLOWS

*Symptoms.*—Carrot (*Daucus carota* var. *sativa*) plants naturally infected with yellows showed a marked yellowing of the younger central leaves, while the older outer leaves were usually reddened or purple. The discoloration of the older outer leaves often failed to develop in carrots experimentally infected with the disease in the greenhouse. The

younger central leaves were dwarfed and the petioles sometimes twisted (figs. 1, 2) ; occasionally a dense growth of adventitious chlorotic shoots developed at the center of the crown (figs. 3, 4). The leaflets on the

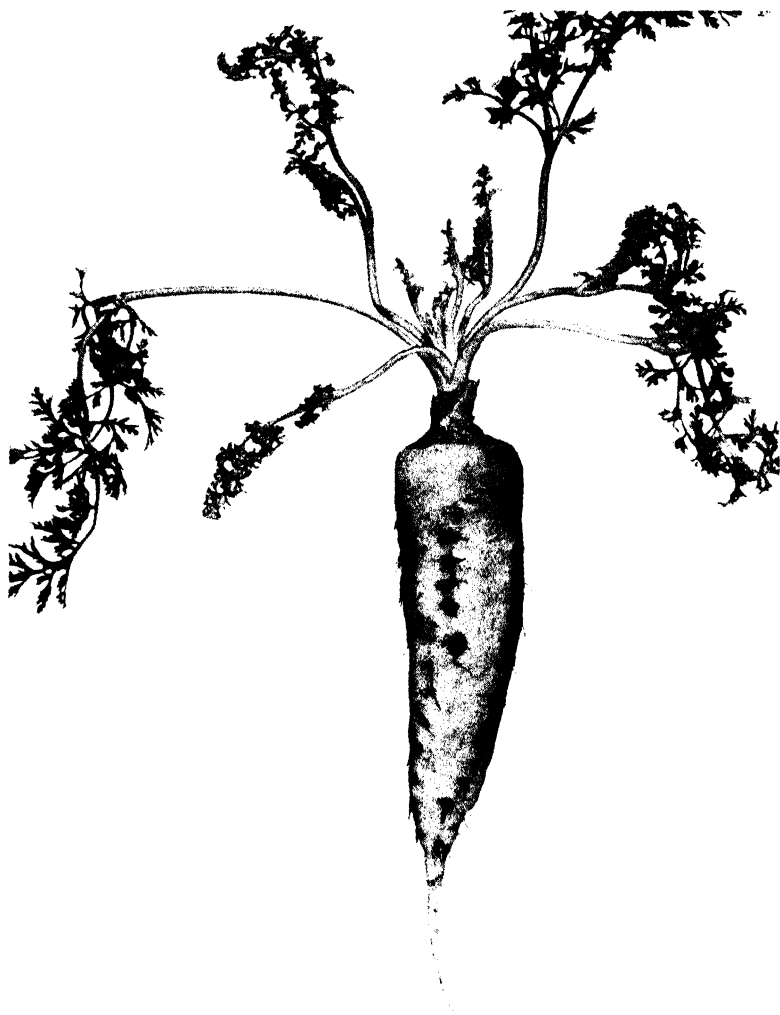


Fig. 1. White Belgian carrot naturally infected with yellows, showing dwarfed central leaves with some of the petioles twisted.

shortened petioles were sometimes reduced to short filaments, which often became dry. Carrot plants experimentally infected with yellows sometimes developed a short central seed stalk or several seed stalks,

and a constriction sometimes occurred below the crown of the carrot. Carrots in an advanced stage of the disease showed numerous bunched rootlets arising from elevations on the carrot root (figs. 1, 3).



Fig 2. Short White carrot naturally infected with yellows, showing twisted petioles in a top view.

*Incubation Period* —The incubation period of the disease was determined in 3 white, 1 yellow, and 7 orange varieties of carrots. The length of time that elapses from the inoculation of the plant by infective leafhoppers until the youngest leaves became chlorotic or the

petioles began to twist was considered as the incubation period of the disease. Carrots used as a check or control remained healthy.

In two experiments small and large carrots were experimentally infected with yellows by the leafhoppers reared on naturally infected celery. The minimum, maximum, mean, and average incubation periods in varieties of carrots are given in table 1.

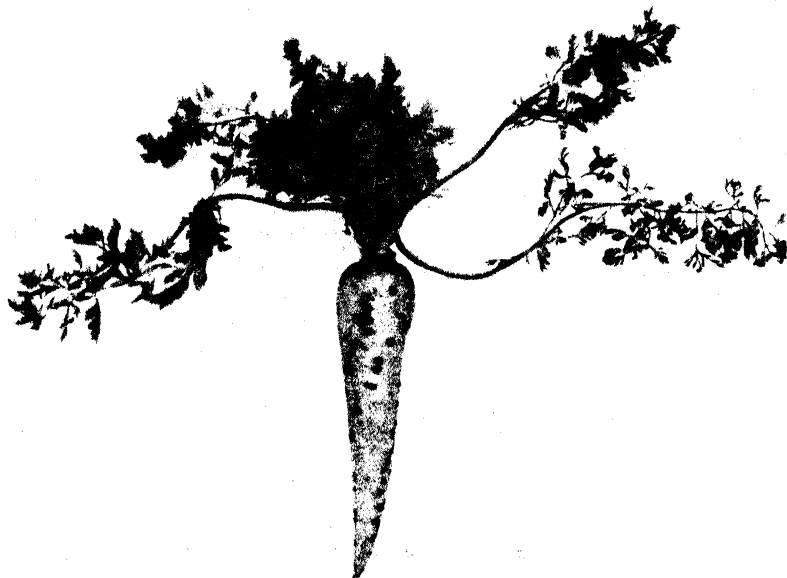


Fig. 3. White Belgian carrot naturally infected with yellows, showing dense growth of adventitious shoots at the center of the crown.

A comparison of the averages in table 1 shows that the incubation period is shorter in small (young) carrots than in large ones. In all probability large carrots are more resistant to the disease. Similar results were obtained with curly top of sugar beets<sup>(4)</sup>—the smaller the beet the shorter the incubation period of the disease.

The incubation period of the disease in carrots infected by previously noninfective leafhoppers which had fed on naturally infected asters is shown in table 2. The minimum incubation period varied from 15 to 29 days with an average of 22.7 days. The maximum incubation period ranged from 34 to 76 days with an average of 46.5 days. The average percentage of infection of the white varieties of carrots was 65.2, of the yellow 18.7, and of the orange 51.1. The Yellow Belgian carrot was most resistant to the disease.

TABLE 1

INCUBATION PERIOD OF YELLOW'S DISEASE IN CARROTS INFECTED BY *CICADULA*  
DIVISA BRED ON NATURALLY INFECTED CELERY

Variety	Plants inoculated	Leaf-hoppers on each plant	Plants infected	Plants healthy	Incubation period in plant, days	
					Range	Mean
Small (young) carrots						
<i>White varieties:</i>						
Short White.....	5	25	2	3	19-20	19.5
White Mastodon.....	4	25	1	3	103	.....*
White Belgian.....	2	25	2	0	18-22	20.0
<i>Yellow varieties:</i>						
Yellow Belgian.....	2	25	0	2	.....	.....
<i>Orange varieties:</i>						
Chantenay.....	2	25	2	0	20-21	20.5
Danvers Half Long.....	4	25	4	0	21-41	31.0
Early Scarlet Horn.....	2	25	2	0	21-31	26.0
French Forcing.....	4	25	2	2	19-22	20.5
Long Orange.....	2	25	1	1	25	.....*
Nantes.....	2	25	0	2	.....	.....
Oxheart or Guerande.....	2	25	1	1	48	.....*
Total.....	31	.....	17	14	.....	.....
Average.....	.....	25	.....	.....	.....	22.9
Large carrots						
<i>White varieties:</i>						
Short White.....	2	25	1	1	21	.....*
White Mastodon.....	2	25	0	2	.....	.....
White Belgian.....	2	25	1	1	23	.....*
<i>Yellow varieties:</i>						
Yellow Belgian.....	6	25	1	5	21	.....*
<i>Orange varieties:</i>						
Chantenay.....	6	25	2	4	26-35	30.5
Danvers Half Long.....	2	25	0	2	.....	.....
Early Scarlet Horn.....	6	25	3	3	28-56	42.0
French Forcing.....	2	25	0	2	.....	.....
Long Orange.....	2	25	1	1	47	.....*
Nantes.....	3	25	0	3	.....	.....
Oxheart or Guerande.....	6	25	2	4	26-88	57.0
Total.....	39	.....	11	28	.....	.....
Average.....	.....	25	.....	.....	.....	43.2

\* No mean given because only one plant became infected.

*Recovery of Virus.*—The transmission of yellows by previously non-infective leafhoppers from orange varieties of carrots naturally infected with the disease to healthy asters and celery is shown in table 3. Five lots of 20 noninfective leafhoppers each were exposed for a period of 2 days on 5 carrot-yellows plants, one lot to a plant, and then 10



Fig. 4. Short White carrot naturally infected with yellows, showing central adventitious shoots with outer leaves removed.

insects of each lot were exposed for 20 days to a healthy aster, and 10 insects of each lot to a healthy celery plant. Each lot was then transferred to another healthy aster or another healthy celery plant and kept on these plants for a period of 7 days. A third set of celery plants were exposed for a period of 7 days to the leafhoppers which had previously been fed on the second set of celery plants, except one lot of insects, which had died.

TABLE 2

INCUBATION PERIOD OF YELLOWS DISEASE IN CARROTS INFECTED BY PREVIOUSLY  
NONINFECTIVE *CICADULA DIVISA* FED ON NATURALLY INFECTED ASTERS

Variety	Plants inoculated	Leaf- hoppers on each plant	Plants infected	Plants healthy	Incubation period in plant		
					Minimum	Maximum	Mean
<i>White varieties:</i>					<i>days</i>	<i>days</i>	<i>days</i>
Short White.....	7	25	5	2	21	45	32.4
White Mastodon.....	8	25	4	4	25	42	36.7
White Belgian.....	8	25	6	2	23	76	43.2
<i>Yellow varieties:</i>							
Yellow Belgian.....	16	25	3	13	26	40	34.7
<i>Orange varieties:</i>							
Chantenay.....	4	25	3	1	29	36	29.3
Danvers Half Long.....	7	25	3	4	23	37	29.7
Early Scarlet Horn.....	5	25	4	1	22	49	35.5
French Forcing.....	7	25	4	3	25	40	33.3
Long Orange.....	9	25	3	6	20	34	26.7
Nantes.....	8	25	2	6	15	72	43.5
Oxheart or Guerande.....	5	25	4	1	21	41	30.7
Total.....	84	.....	41	43	.....	.....	.....
Average.....	.....	25	.....	.....	22.7	46.5	34.2

TABLE 3

TRANSMISSION OF YELLOWS BY PREVIOUSLY NONINFECTIVE *CICADULA DIVISA* FROM  
CARROTS NATURALLY INFECTED WITH THE DISEASE TO  
HEALTHY ASTERS AND CELERY\*

Carrot plant No.	Results with asters		Results with celery		
	First set	Second set	First set	Second set	Third set
1.....	+	+	+	+	+
2.....	-	-	+	+	†
3.....	+	-	-	-	-
4.....	+	+	+	+	+
5.....	+	-	+	+	+
Total positive (+).....	4	2	4	4	3
Total negative (-).....	1	3	1	1	1

\* The plus sign (+) indicates the production of the disease, and the minus sign (-) shows that no disease resulted.

† Insects died.

It is evident from table 3 that 10 leafhoppers which fed on carrot plant No. 2, failed to transmit yellows to either of the asters, while 10 insects which fed on the same diseased carrot transmitted yellows to 2 successive celery plants. The third plant was not tried with this lot of leafhoppers because the insects died. In the case of carrot plant No. 3,

yellows was transmitted to the first aster but not to the second. Another group of 10 leafhoppers which fed on the same diseased carrot failed to transmit yellows to 3 successive celery plants.

In similar tests, previously noninfective leafhoppers, after feeding on Short White and White Belgian carrot plants naturally infected with yellows, transmitted the disease to asters, Erfurt Giant celeriac (*Apium graveolens* var. *rapaceum*), and the following varieties of celery: Golden Self-Blanching, Large Smooth Prague, and Rosy Plume (*Apium graveolens* var. *dulce*).

Successive inoculations were made from each variety of carrot experimentally infected with the disease to celery or asters or both and back to carrots. Previously noninfective leafhoppers after feeding on carrot plants infected with yellows often transmitted the disease to celery and asters but numerous trials were required to transfer the disease from infected celery or asters back to carrots. Repeated lots of leafhoppers were often used in the transfer of yellows from infected celery or asters back to carrots. Better results were obtained in the transmission of the disease when small carrot plants were used. The disease was not transmitted from inoculated carrots which failed to show symptoms of the disease.

The transmission of the disease from carrots experimentally infected with yellows to the same variety of carrot also required numerous trials. The leafhoppers on healthy carrots often died before the incubation period of the virus was completed in the insects. It was found, however, that the males lived longer on diseased than on healthy carrots, and hence the insects were kept on diseased carrots for a period of 2 to 3 weeks so that the incubation period was completed before transferring them to healthy carrots. Females lived longer than males on carrots, and hence females were allowed to deposit their eggs in the petioles of diseased carrots, and after the nymphs hatched and fed for a period of about three weeks they were transferred to healthy carrots.

*Inoculations of Healthy Celery by Means of Leafhoppers Fed on Filtered Juice from Carrots Infected with Yellows.*—All attempts to transmit yellows by feeding noninfective *Cicadula divisa* on the filtrate prepared from carrots infected with yellows and then transferring the insects to healthy celery were failures. The juice was extracted from a total of 15 Short White and White Belgian carrots naturally infected with yellows and filtered through coarse and fine Berkefeld candles. The leafhoppers after feeding on the filtrate for a period of 3 hours were transferred to 56 healthy celery plants but without effect. The filtrate was a favorable food for the leafhoppers.



*Longevity and Life Cycle of Cicadula Divisa on Carrots.*—The longevity of the last living male on each variety of carrot during the spring, summer, and autumn is shown in table 4. Most of the males died a few days after being exposed to healthy varieties of carrots. The mortality was higher on small carrots than on large ones. After the plants developed symptoms of yellows the insects lived longer. It is possible that the virus produces changes in the sap of the carrot which are of some biological significance to the insect, as was suggested in a previous paper.<sup>(5)</sup> The leafhopper completed its life cycle on all varieties of carrots infected with yellows except Yellow Belgian. A low population acquired the adult stage on most varieties of carrots.

TABLE 4

LONGEVITY OF LAST LIVING MALE *CICADULA DIVISA* ON VARIETIES OF CARROTS

Variety	Longevity of males		
	Minimum	Maximum	Average
<i>White varieties:</i>	<i>days</i>	<i>days</i>	<i>days</i>
Short White .....	5	14	9.0
White Mastodon .....	2	30	10.5
White Belgian .....	4	16	7.7
<i>Yellow varieties:</i>			
Yellow Belgian .....	3	26	9.5
<i>Orange varieties:</i>			
Chantenay .....	2	25	7.7
Danvers Half Long .....	2	10	5.2
Early Scarlet Horn .....	4	28	15.4
French Forcing .....	8	26	14.2
Long Orange .....	7	30	12.5
Nantes .....	4	31	13.0
Oxheart or Guerande .....	2	31	9.6

## YELLOWS OF HAMBURG OR TURNIP-ROOTED PARSLEY

Hamburg or Turnip-rooted parsley (*Petroselinum hortense* var. *radicosum*) naturally or experimentally infected with yellows showed a dense growth of chlorotic leaves at the center of the crown (fig. 5). These leaves were dwarfed with upright petioles frequently twisted (fig. 6). The symptoms in experimentally infected varieties of parsley were similar to those observed in the field.

Twelve Hamburg or Turnip-rooted parsley plants were inoculated with from 5 to 25 infective *Cicadula divisa* and six plants developed typical symptoms of yellows. Table 5 shows that the incubation period

of the disease in Hamburg parsley varied from 36 to 106 days, with an average of 65.2 days. The longer incubation periods were obtained with Hamburg parsley infected during the winter.



Fig. 5. Hamburg or Turnip-rooted parsley naturally infected with yellows, showing a dense growth of dwarfed leaves at the center of the crown.

TABLE 5

INCUBATION PERIOD OF YELLOWS DISEASE IN HAMBURG OR TURNIP-ROOTED PARSLEY INFECTED BY *CICADULA DIVISA*

Plants inoculated	Leaf-hoppers on each plant	Plants infected	Plants healthy	Incubation period in plant
				<i>days</i>
1	15	1	0	36
1	10	1	0	42
1	10	1	0	49
1	10	1	0	56
1	5	1	0	102
1	15	1	0	106
6	25	0	6	.....
Total: 12	.....	6	6	.....
Average	.....	.....	.....	65.2

Successive inoculations were made from Hamburg parsley naturally and experimentally infected with yellows to celery or asters and from these plants back to the same variety of parsley. The disease was also

transmitted from infected Hamburg parsley to the same variety of parsley, to Short White, and White Belgian carrots, and from the white varieties of carrots back to Hamburg parsley.

The leafhopper completed its life cycle on Hamburg or Turnip-rooted parsley and a large population of adults was obtained.



Fig. 6. Leaves showing curved petioles from Hamburg or Turnip-rooted parsley naturally infected with yellows.

#### YELLOWS OF SINGLE OR PLAIN PARSLEY

The symptoms of the disease in Single or Plain parsley (*Petroselinum hortense*) experimentally infected with yellows were somewhat similar to those of Hamburg or Turnip-rooted parsley. The youngest leaves were yellow and the upright petioles were often twisted. Secondary chlorotic shoots sometimes developed.

Thirteen plants were inoculated by from 10 to 25 infected leafhoppers and seven plants developed typical symptoms of yellows. The incubation period of the disease varied from 36 to 58 days with an average of 48.7 days as shown in table 6.

The virus was recovered by previously noninfective leafhoppers from experimentally infected Single or Plain parsley, and yellows was transmitted to healthy celery and asters and from these plants back to the same variety of parsley.

A low population of adult leafhoppers was bred on this variety of parsley after symptoms of yellows developed.

TABLE 6  
INCUBATION PERIOD OF YELLOWS DISEASE IN SINGLE OR PLAIN PARSLEY  
INFECTED BY *CICADULA DIVISA*

Plants inoculated	Leaf-hoppers on each plant	Plants infected	Plants healthy	Incubation period in plant
				days
1	25	1	0	36
1	25	1	0	38
1	10	1	0	40
1	15	1	0	56
1	25	1	0	56
1	25	1	0	57
1	20	1	0	58
6	10-25	0	6	...
Total: 13	...	7	6	...
Average	...	...	...	48.7

#### YELLOWS OF DOUBLE CURLED, EXTRA TRIPLE CURLED, AND FERN LEAF OR MOSS CURLED PARSLEY

These three varieties of parsley (*Petroselinum hortense* var. *crispum*) experimentally infected with yellows showed dwarfed, chlorotic, innermost leaves sometimes with twisted or curled petioles.

The incubation period of the disease in the three varieties of parsley is given in table 7. Twelve Double Curled parsley plants were inoculated but only one plant developed symptoms of yellows. The incubation period of the disease was 37 days. The incubation period of the disease in Triple Curled parsley varied from 32 to 39 days with an average of 37 days, in Fern Leaf or Moss Curled parsley from 39 to 50 days, with an average of 44.6 days.

Cross-inoculations from the three varieties of parsley showing symptoms of yellows to celery and asters were failures.

The longevity of the last living male and female leafhopper on the three varieties of parsley is shown in table 8. The average adult life of

the females was longer than the males. The life cycle was not completed on the three varieties of parsley.

Nymphs which hatched from eggs deposited in the three varieties of parsley failed to complete their life cycle in the greenhouse.

TABLE 7

INCUBATION PERIOD OF YELLOW'S DISEASE IN VARIETIES OF PARSLEY INFECTED  
BY *CICADULA DIVISA*

Variety	Plants inoculated	Leaf-hoppers on each plant	Plants infected	Plants healthy	Incubation period in plant
Double Curled.....	1 11	10 10-60	1 0	0 11	days 37
Total.....	12	...	1	11	...
Extra Triple Curled.....	1 1 1 1 1 11	25 25 10 25 25 10-30	1 1 1 1 1 0	0 0 0 0 0 11	32 36 39 39 39 ...
Total.....	16	...	5	11	...
Average.....	...	...	...	...	37.0
Fern Leaf or Moss Curled.....	1 1 1 1 1 1 1 14	25 25 25 25 25 25 25 25-45	1 1 1 1 1 1 1 0	0 0 0 0 0 0 0 14	39 43 43 43 47 47 50 ...
Total.....	21	...	7	14	...
Average.....	...	...	...	...	44.6

TABLE 8

LONGEVITY IN DAYS OF LAST LIVING MALE AND FEMALE *CICADULA DIVISA* ON THREE  
VARIETIES OF PARSLEY

Variety	Longevity of males, in days			Longevity of females, in days		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Double Curled.....	2	29	11.1	14	20	16.3
Extra Triple Curled.....	3	29	16.0	9	25	17.3
Fern Leaf or Moss Curled.....	2	33	9.1	11	34	19.0

## YELLOW S OF HOLLOW CROWN PARSNIP

Hollow Crown parsnip (*Pastinaca sativa*) naturally infected with yellows showed dwarfed, chlorotic, innermost leaves with twisted petioles (fig. 7). Plants grown from seeds experimentally infected with yellows showed similar symptoms in the greenhouse. The check or control plants remained healthy.



Fig. 7. Hollow Crown parsnip naturally infected with yellows, showing dwarfed leaves and twisted petioles.

The average incubation period of the disease in experimentally infected plants was 40 days.

The virus was recovered by previously noninfective leafhoppers from naturally and experimentally infected parsnip. Yellows was trans-

mitted to healthy celery and asters and from these plants back to healthy parsnips.

Hollow Crown parsnip was a favorable food plant of the leafhopper and the life cycle was completed on this variety of parsnip.

### SUMMARY

Experiments demonstrated that previously noninfective *Cicadula divisa* after feeding on Short White, White Belgian, and orange varieties of carrots, Hamburg or Turnip-rooted parsley, and Hollow Crown parsnip naturally infected with yellows, became infective and transmitted typical yellows to healthy asters and celery. Previously noninfective leafhoppers, after feeding on the experimentally infected asters and celery transmitted the disease back to the same varieties of carrots, parsley, and parsnip.

Eleven varieties of carrots, five varieties of parsley, and one variety of parsnip were experimentally infected with yellows by infective leafhoppers. After symptoms of yellows developed, previously noninfective leafhoppers feeding on all of the experimentally infected varieties of carrots, Hamburg or Turnip-rooted and Single or Plain parsleys, and Hollow Crown parsnip transmitted the disease to healthy asters and celery. The disease was transmitted from infected asters and celery back to healthy carrots, Hamburg or Turnip-rooted, and Single or Plain parsleys, and Hollow Crown parsnip. The virus was not recovered from Double Curled, Extra Triple Curled, and Fern Leaf or Moss Curled parsleys. Yellows disease was also transmitted from infected carrots to healthy ones, and similarly from parsley to parsley, carrot to parsley, and parsley to carrot.

These experiments prove that the virus of carrot, parsley, and parsnip yellows can be transmitted to asters and celery. The virus used in the transmission experiments was obtained from carrots, parsley, and parsnips infected with yellows in the field and from asters and celery infected with yellows under natural conditions. The experiments demonstrate that the virus of carrots, parsley, and parsnip yellows is identical with California aster and celery yellows.

The average incubation period of the disease in small carrots was 22.9 days and in large carrots 43.2 days. In all probability large carrots are more resistant to the disease. The minimum incubation period of the disease in carrots varied from 15 to 29 days, and the maximum incubation period ranged from 20 to 103 days. The incubation period of the disease in Hamburg or Turnip-rooted parsley varied from 36 to 106 days, with an average of 65.2 days. The longer incubation periods were

obtained during the winter. The incubation period of the disease in Single or Plain parsley ranged from 36 to 58 days with an average of 48.7 days. The incubation period in Double Curled parsley was 37 days, the average in Extra Triple Curled was 37 days, and in Fern Leaf or Moss Curled, 44.6 days. The average incubation period of the disease in Hollow Crown parsnip was 40 days.

The leafhopper completed its life cycle on all varieties of carrots except Yellow Belgian. A low population of leafhoppers was obtained on most varieties of carrots. The life cycle was also completed on Hamburg or Turnip Rooted, Single or Plain parsleys, and Hollow Crown parsnip. The insect failed to complete its life cycle on Double Curled, Triple Curled, and Fern Leaf or Moss Curled parsleys.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

NOVEMBER, 1932

No. 4

## INJURIOUS EFFECTS OF MANGANESE AND IRON DEFICIENCIES ON THE GROWTH OF CITRUS<sup>1, 2</sup>

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### INTRODUCTION

The presence of a small amount of manganese is known to be essential for chlorophyll formation in certain plants, while the presence of an excess is destructive to chlorophyll. It is believed that neither iron nor manganese is a constituent of chlorophyll. Iron is the only element commonly spoken of as being a catalyzer of chlorophyll formation. The importance of manganese in chlorophyll catalysis is becoming increasingly more evident, which is believed to be indirectly due to its action upon the iron of the cells. Interest in these elements is increased because very little is known regarding their effects on the growth of citrus, and especially of their possible bearing on the mottle-leaf problem.

With citrus in sand or solution cultures no manganese-deficiency symptoms could be obtained when so-called "chemically pure" iron was used except in cases where a minimum of iron was used with cultures of rapidly growing 2 to 3-year-old trees. Chemical analysis showed that every source of iron available for culture solutions was contaminated with manganese to a greater or lesser extent, and that until manganese-free iron was prepared it was impossible to differentiate between the effects of deficiencies of iron and manganese. In solution cultures with seedlings, in short-term experiments with orange

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<sup>1</sup> Received for publication April 21, 1932.

<sup>2</sup> Paper No. 271, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

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or lemon cuttings, or in longer-term experiments in which larger plants are obtained in solution cultures where generous amounts of iron citrate or tartrate are used, no manganese-deficiency symptoms were evident because of the manganese contaminations in the iron supply.

The writer has always added manganese to culture solutions used for citrus, more as a matter of good practice than for any other reason. With manganese as an unknown contamination in the iron supply it made no difference whether manganese was added or was omitted; hence the belief that its presence was of no consequence.

An investigation was therefore undertaken to determine whether manganese and iron were necessary for healthy growth in citrus, and if so, the symptoms of their deficiencies in artificial solution cultures, their relation to one another, and their possible bearing on the mottle-leaf problem.

## REVIEW OF LITERATURE

In a study of the effect of manganese, copper, and zinc, McHargue,<sup>(17)</sup> McHargue and Shedd,<sup>(19)</sup> and McHargue and Calfee<sup>(18)</sup> have recently reported concentrations of these elements as stimulating greater growth of certain plants.

Manganese deficiency in pure quartz sand cultures of annual plants soon resulted (Miller<sup>(22)</sup>) in a chlorotic condition, but healthy growth was quickly resumed upon adding manganese sulfate. Samuel and Piper<sup>(24)</sup> concluded that gray speck disease of oats is due to a deficiency of manganese and made the suggestion that possibly pecan rosette, mottle-leaf of citrus, and walnut yellows are manganese-deficiency diseases.

Bishop<sup>(1)</sup> reported that when the manganese supply was exhausted, the plants he used either died or remained dwarfed. A manganese deficiency caused yellow spots on the new leaves, and the leaves were unable to synthesize chlorophyll. Chlorosis or yellow spots also occurred when an excess of manganese was used. Low as well as high manganese caused a loss of chlorophyll. He was unable to confirm the results of Johnson,<sup>(12)</sup> who found that manganese depressed the iron assimilation. Bishop<sup>(1)</sup> confirmed the results obtained by Kelley,<sup>(13)</sup> who found that manganese increases the calcium in plants, which supposedly counteracts the toxicity of the high manganese concentrations. The chlorosis of pineapple leaves on plants grown on manganese soils is considered by McGeorge<sup>(16)</sup> to be due to a greater assimilation of lime, indirectly caused by the presence of manganese in excessive amounts in the soil.

McLean,<sup>(20)</sup> McLean and Gilbert,<sup>(21)</sup> and Gilbert and McLean<sup>(5)</sup> report the curing of lime-induced chlorosis of spinach and other plants by feeding the plants manganese through the stomata. External applications of copper and manganese on certain chlorotic plants of the Florida Everglades have been found by Bryan<sup>(2)</sup> to have a stimulating effect upon growth. The application of manganese to the soil has corrected chlorosis of soy beans in various soil types on the lower Coastal Plain of North Carolina (Willis<sup>(26)</sup> and Mann<sup>(15)</sup>). Lee and McHargue<sup>(14)</sup> found that Pahala-blighted leaves of sugar cane had a greatly reduced manganese content but no decrease of iron. An increase in soil acidity, the addition of manganese to the soil, or the application of manganese to the leaves soon resulted in recovery of the blighted leaves.

Manganese is essential, but will not replace iron in the growth of *Chlorella* sp., according to Hopkins.<sup>(9)</sup> The function of manganese is suggested as being that of a controlling agent in maintaining a suitable ratio of ferrous to ferric iron in the culture or in the cell. He found that in vitro, manganese tends to prevent the reduction of ferric to ferrous iron by sodium citrate. Cultures of yeasts indicated that the reduction of the iron by the yeast organism tends to be prevented by the presence of manganese. Sufficient manganese is considered essential in order to insure the reoxidation of the iron after its reduction by the organism. Excessive manganese either results in too high a concentration of ferric ions or prevents the reduction to the ferrous state by the organism. Ingalls and Shive<sup>(11)</sup> have reported the distribution of iron in plants as being related to the H-ion concentration of the tissue fluids.

In their studies on the growth of *Lemna* it was found by Clark and Fly<sup>(3)</sup> that manganese was not essential in the nutrition of *Lemna major*. Since the appearance of their paper, Hopkins<sup>(10)</sup> has shown that manganese is an essential element for healthy growth of *Lemna*. Richards<sup>(23)</sup> has reported that soil conditions have little to do with the manganese content of foodstuffs.

The toxic action of an excess of manganese on citrus in sand culture has recently been studied by Haas<sup>(6)</sup>. Mottling as well as chlorosis resulted, and in severe cases a characteristic gum or resin spot appeared in the leaves.

## METHODS

The present work employed rooted cuttings of Rough lemon, lemon (*Citrus limonia* Osbeck), and orange (*Citrus sinensis* Osbeck) in solution cultures. These were grown in the glasshouse in shallow enameled pans through a period of 12 or more months. Budded citrus trees in large sand cultures were used to supplement experiments conducted with solution cultures.

The cuttings were grown in Hoagland's solution modified so as to contain double the concentration of calcium nitrate. The concentration of ions in this solution, in parts per million, was as follows:

Na	K	Ca	Mg	NO <sub>3</sub>	Cl	SO <sub>4</sub>	PO <sub>4</sub>	Total
7	185	318	54	1,211	10	216	105	2,106

Two and one-half p.p.m. of zinc as zinc nitrate and 0.1 p.p.m. of boron as boric acid were used in all cultures. To this solution were added various ions such as iron, manganese, silicic acid, etc., as the experiments required. Manganese was omitted in some cultures and iron in others.

"A-Y"<sup>4</sup> was used as a source of manganese in certain cultures, while in others this same solution was used with manganese omitted. Manganese-free iron was prepared electrolytically with an apparatus that consisted of a glass battery jar in which was placed a porous clay cell or cylinder. A bar of iron placed in the clay cell served as the positive electrode, while a heavy platinum wire fused into a glass tube containing mercury served as the negative electrode. Iron sulfate solution prepared in a manner similar to that of Samuel and Piper<sup>(24)</sup> filled the jar and clay cell. An auto battery served as a source of direct current. In this way, a large quantity of manganese-free iron citrate was prepared.

It was found to be more beneficial to the plant to renew the evaporated water daily than to keep the level of the solution constant. A

<sup>4</sup> "A-Z" stock solution contains the following amounts of salts in 18 liters: 15.9408 grams Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> · 18H<sub>2</sub>O, 1.6953 grams KI, 5.1787 grams Ti<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, 1.9300 grams KBr, 4.1959 grams Sr(NO<sub>3</sub>)<sub>2</sub> · 4H<sub>2</sub>O, 22.9709 grams LiNO<sub>3</sub> · 3H<sub>2</sub>O, 5.2628 grams MnSO<sub>4</sub> · 4H<sub>2</sub>O, 7.3071 grams H<sub>3</sub>BO<sub>3</sub>, and 5.7503 grams NH<sub>4</sub>NO<sub>3</sub>. When 25 cc of "A-Z" stock solution is used in 18 liters of Hoagland's solution, a concentration of 0.1 p.p.m. of the following is obtained: aluminum, iodine, titanium, bromine, strontium, lithium, manganese, boron, and ammonium. The distilled water is obtained from a tin-lined copper still and stored in a copper tank lined with an electrolytic covering of tin. It is possible, therefore, that the distilled water contains an extremely small amount of copper. Additional copper was therefore not added to the culture solution. When lithium is omitted "A-Z" is referred to as "A-Y."

leveling device (see Haas,<sup>(6)</sup> page 487) was used only when it was not possible to fill the culture vessel during a 24-hour period. No aeration of the culture solution was carried on except for the renewal of oxygen



Fig. 1. Rough-lemon cutting in a culture solution to which were added manganese-free iron, zinc, silicic acid, and "A-Y." The character of the growth indicates the suitability of solution cultures for nutrition deficiency studies.

added in the distilled water containing iron. The refilling of the culture vessels daily prevented undue salt-concentration effects and also retarded the warming of the solution during periods of high temperature. Culture pans are now being sunk in sand that can be kept moist in shallow galvanized-iron pans.

## EFFECTS OF CULTURE SOLUTIONS ON GROWTH

Rough-lemon, lemon, and orange cuttings, because of their rapid growth, proved to be well adapted to experiments involving manganese or iron. Figure 1 illustrates the splendid growth typical of many Rough-lemon cuttings that were grown in the modified Hoagland's culture solu-

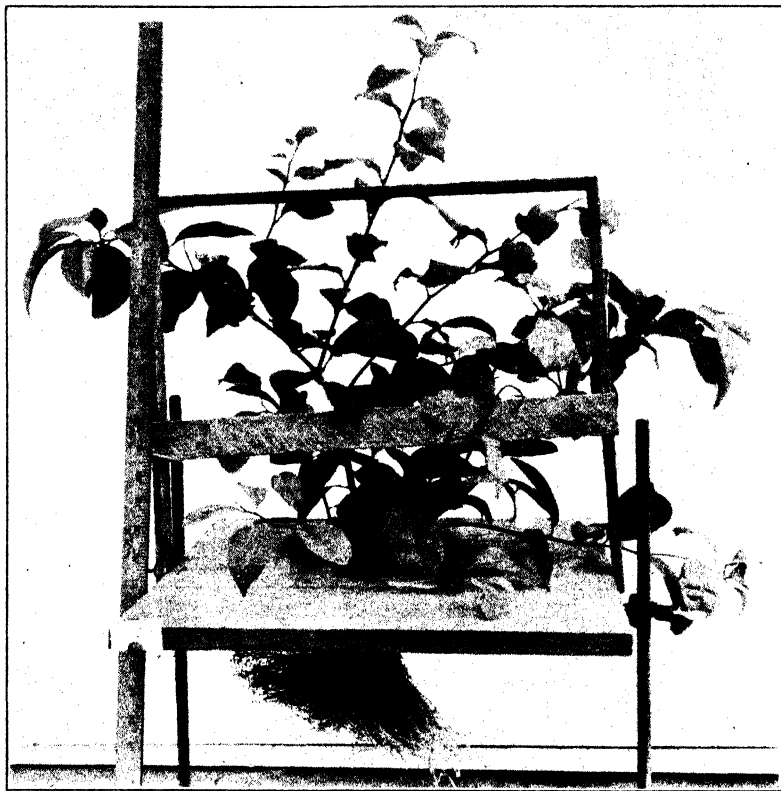


Fig. 2. Growth of Rough-lemon cuttings in a culture solution to which purified iron, zinc, and manganese-free "A-Y" were added. Several young shoots have lost all their young leaves and have died. See figure 3.

tion to which was added manganese-free iron, zinc, silicic acid, and "A-Y." Several parts per million of manganese-free iron were added two to three times each week.

Cuttings in solution cultures have successfully withstood maximum glasshouse temperatures as high as 109°F and culture-solution temperatures as high as 91°F. The cutting shown in figure 1 transpired 18.5

liters during 19 days when the daily maximum glasshouse temperature varied from 92° to 100° and the solution temperature from 71° to 82°. Another cutting transpired 33.0 liters during 45 days when the daily maximum glasshouse temperature varied from 91° to 109° and the day-time solution temperature from 73° to 91°. The transpiration per unit area was not calculated, but the figures indicate the absorptive capacity of the root system. The roots subjected to such temperatures retained a healthy white color, as may be seen in figure 1; the leaves were dark green and the shoots vigorous.

*Effects of Manganese Deficiency on Growth of Rough Lemon.*—When manganese was omitted from the culture solution the growth of Rough-lemon cuttings was severely restricted and there was a marked abscission of young leaves that were unable to attain full size. Figures 2 and 3 show two Rough-lemon cuttings 6 months after they were transferred from the control solution to one lacking manganese. The leaves were yellowish-green (chlorotic), as though they were in need of iron, even though manganese-free iron was added two or three times a week.

This might be taken to indicate, as Hopkins has suggested, that the leaves were unable to reoxidize the iron after its reduction by the plant. Data given later show that a deficiency of manganese did not prevent the leaves from obtaining large amounts of iron, but it is likely that the iron was of little use to the leaves for the formation of chlorophyll. It is striking that during the months of manganese deficiency the roots remained in an excellent condition, ready to start growth upon the addition of manganese to the culture solution.

*Recovery of Growth of Rough Lemon upon Addition of Manganese to Manganese-deficient Cultures.*—The quick response of the growth of manganese-deficient Rough-lemon cuttings upon the addition of manganese may be seen in figures 4 and 5. The addition of 1 p.p.m. of manganese as manganese sulfate to the culture solution was followed by a new cycle of growth (fig. 5) almost simultaneously throughout the entire top. The new leaves were deep green in color.

*Effects of Manganese Excess on Growth of Rough Lemon and on Orange.*—Chlorosis in citrus was produced not only by a deficiency of manganese, but also by an excess. Chlorosis of leaves of Rough-lemon cuttings occurred when as little as 5 p.p.m. of manganese was added two or three times a week. The excessive manganese made the roots dark brown, and the new leaves became successively more chlorotic with each new cycle of growth (fig. 6). Here chlorosis is associated with excessive manganese even when generous supplies of manganese-free iron were added. The culture solution was then renewed and the total manganese concentration was limited to 5 p.p.m. Iron additions were





Fig. 3. Growth of Rough-lemon cuttings in a culture solution to which purified iron, zinc, and manganese-free "A-Y" were added. This plant was larger than that shown in figure 2 when the cuttings were removed from the control solution and placed in the manganese-free solution. Then for 6 months, growth of the tops was at a standstill. The roots, however, maintained a white healthy appearance during this period.



Fig. 4. Rough-lemon cutting after 6 months in a culture solution containing iron but no manganese. The manganese-deficient leaves of the last growth cycle were removed for analysis just prior to the addition of the manganese to the culture solution. Figure 5 shows the same cutting after manganese had been supplied.



Fig. 5. Same cutting as in figure 4, 29 days after manganese was supplied.

continued as before. The new root growth was white in contrast to the dark-brown older roots and the chlorotic leaves greened up somewhat, but not entirely. Evidently the excessive manganese in the top prevented the iron from functioning in the maintenance of a sufficient concentration of chlorophyll in the leaves.



Fig. 6. Rough-lemon cutting grown in a culture solution to which boron, purified iron, zinc, and manganese sulfate were added. Five p.p.m. of manganese were added two or three times a week.

Manganese excess on young orange trees in sand cultures not only brought about degrees of mottling, chlorosis, and burn, but also produced gum or resin spots on the leaves, as shown in figure 7.

*Effects of Manganese Deficiency on Growth of Eureka Lemon.*—Eureka-lemon cuttings were unable to produce healthy growth when in a culture solution lacking manganese. Figure 8A shows the spotted

leaves in the center of the top and the lack of new growth. The roots were in an excellent condition. Some of the leaves were then removed for analysis. Twenty-nine days after the culture was given manganese, the growth was as seen in figure 8B.

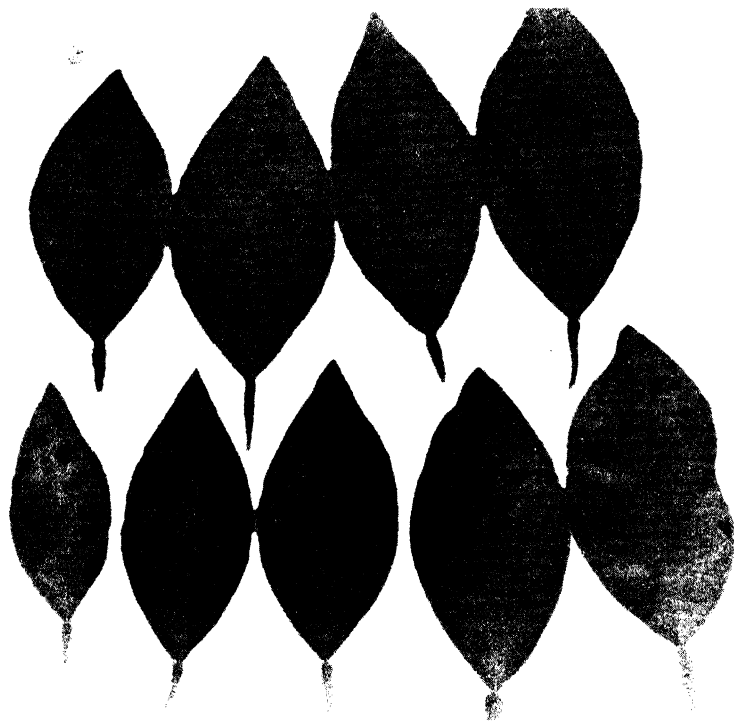
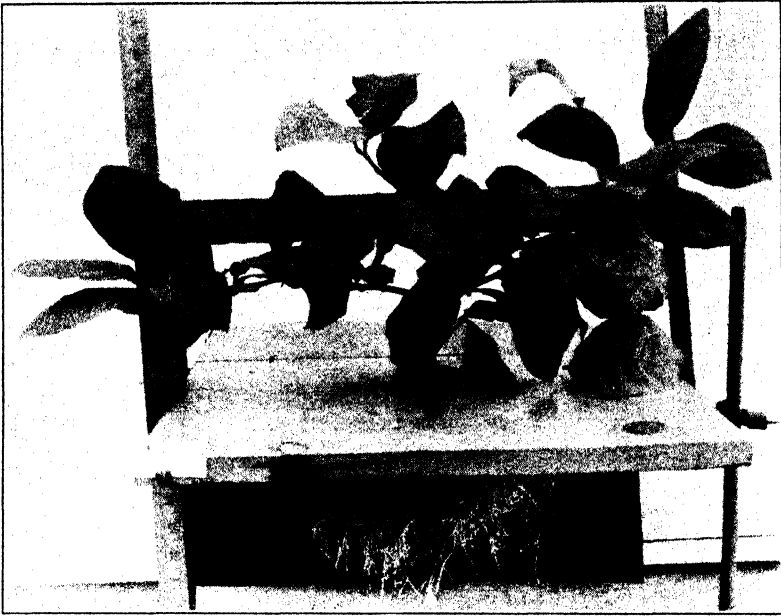


Fig. 7. Dark-green Valencia orange leaves showing raised resinous areas as one effect of excessive manganese. Upper row: ventral leaf surface; lower row: dorsal leaf surface. The leaves were collected from trees in sand cultures that received Hoagland's solution modified so as to contain 100 p.p.m. of manganese as manganese sulfate until toxicity was evident, after which the manganese concentration was reduced to 0.1 p.p.m.

The addition of 0.1 p.p.m. of manganese brought about growth which manganese-free iron was unable to do. Eureka-lemon cuttings in a manganese-free culture solution apparently continue to grow until the available manganese within the plant becomes inadequate for the new leaves to reach full size at maturity. Leaves that attain full size before growth ceases are spotted, and yellowish green; they are not prematurely abscissed. When leaves are unable to reach full size they absciss prematurely. Practically all of the retained leaves of the last growth cycle are unhealthy.



A



B

Fig. 8. Eureka-lemon cuttings: *A*, after 6 months in a culture solution containing iron but no manganese; *B*, same cutting 29 days after manganese was supplied. The manganese-deficient leaves of the last growth cycle in *A* were removed for analysis just prior to the addition of the manganese to the culture solution.

Dark-green, healthy growth can be retained in successive growth cycles when manganese is not deficient, as seen in the control Eureka-lemon cutting shown in figure 9. Each of several cuttings such as that



Fig. 9. Control Eureka-lemon cutting grown in a culture solution containing boron, zinc, purified iron, and 0.1 p.p.m. manganese.

shown in figure 9 were carrying three or four lemons of unusually large size.

The leaves of manganese-deficient Eureka-lemon cuttings become chlorotic, and the shoots bearing them are usually dwarfed. Plate 4, figure 1 shows a branch of a Eureka-lemon cutting grown in a manganese-free culture solution. The new growth following the omission of manganese is chlorotic and the leaves are spotted. Many of the old leaves on the branch are dark green because the culture had previously been supplied with iron and manganese in order to bring the cuttings into excellent condition prior to the manganese-deficiency experiment.

The absence of manganese produces characteristic effects on both leaves and shoots of lemon and Rough-lemon cuttings. These effects consist of curling and premature abscission of young leaves that are

unable to attain full size. In plate 1, figure 1, the second shoot from the right is a young Eureka-lemon shoot taken from a cutting grown in a culture solution containing manganese. The young leaves of this control shoot soon were well expanded, both halves of a leaf being in the same plane and of a healthy color. The other shoots shown in plate 1, figure 1 were taken from several Eureka-lemon cuttings grown in a culture solution deficient in manganese. Even the extremely young leaves are seen to be either curved ventrally along the midrib or the apical region is recurved ventrally. The manganese-deficient leaves are unhealthy in appearance, and a slow death of the defoliated shoot usually follows the abscission of the leaves.

*Nature of Manganese-Deficiency Gum Spots on Lemon Leaves and Shoots.*—The shoots from which immature leaves of cuttings grown in manganese-deficient cultures have abscised are of interest because of the formation of gum that may take place on them. To the right in plate 1, figure 2 are shown two such defoliated Rough-lemon shoots. Such shoots frequently are curved and in the figure show a gummy exudate just above a petiole scar and another midway between two such petiole scars. The characteristic curve of the dead shoots is also shown in figure 2. Shoots killed as a result of high salt concentration usually do not show this curvature.

In plate 1, figure 2 are also shown other Rough-lemon shoots and a Eureka-lemon shoot that were severely defoliated when the cuttings were grown for 6 months in a manganese-deficient culture solution. The terminal portion of the Eureka-lemon shoot has a dead tip. It is of unusual interest that this portion has a gummy or resinous excrecence along the surface like that found in cases of exanthema, or die-back, on citrus in the field. Gum blisters or the orifices of blisters that have already exuded gum may be seen on the Rough-lemon shoots. In exanthema the gum blisters are usually found in the region of petiolar attachment, while here they are scattered indiscriminately along the shoot.

The gum blisters occur in the region of the young xylem, the cambium, and the phloem tissues. Some blisters may enlarge and not break open while others force a passage of the gum to the surface of the shoot. Figure 10 shows a cross section of a gum blister on a shoot of a Rough-lemon cutting grown 6 months in a culture solution lacking manganese. The passageway of the gum from the gum pocket to the surface of the shoot is seen to be a direct one. Neither the vessels nor the wood parenchyma cells are plugged with gum.

The effect of manganese deficiency is evident not only in the young shoots and immature leaves that absciss, but also in the spotted leaves



Fig. 1. Growth of young Eureka-lemon shoots taken from cuttings. The control, the second shoot from the right, was taken from a cutting grown 6 months in a culture solution containing boron, zinc, purified iron, and manganese. One of the leaves was broken off in covering the shoots with glass in taking the photograph. The other shoots were taken from cuttings grown in culture solutions similar to the control, but lacking manganese. Many of the leaves of these manganese-deficient shoots have curled and abscised. The small spots on all leaves represent the oil glands, which are not affected by a manganese deficiency.



Fig. 2. Defoliated shoots taken from cuttings grown 6 months in a culture solution containing zinc, purified iron, and "A-Y" lacking manganese. The two Rough-lemon shoots to the right show internodal exudates of gum. The third, fourth, fifth, and sixth pieces from the right represent one Eureka-lemon shoot. Note the resinous excrescence near the tip. The next two pieces represent one shoot, and the four pieces to the left another shoot taken from Rough-lemon cuttings. The black lines point to gum pockets.



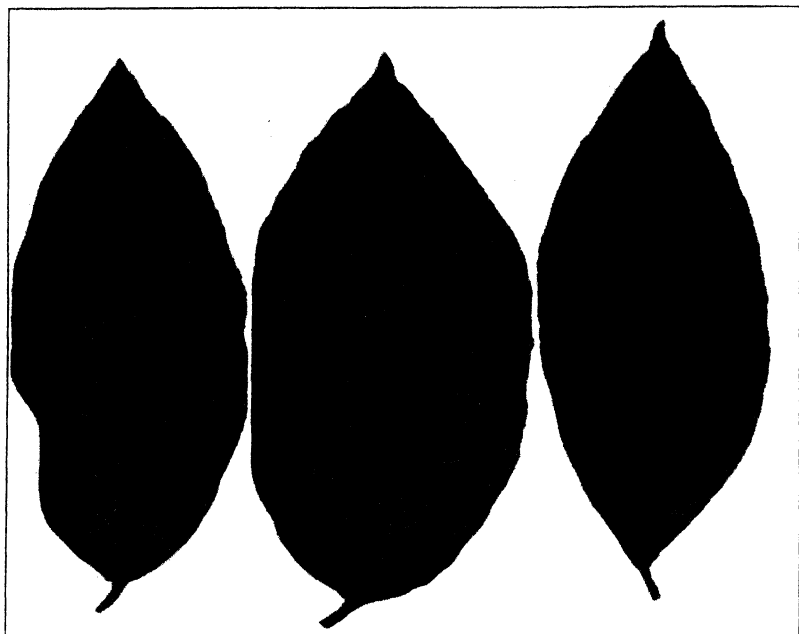


Fig. 1. Eureka-lemon leaves taken from cuttings grown 6 months in a culture solution containing boron, zinc, and purified iron, but no manganese. The leaf on the extreme right was taken from a control cutting that was supplied with manganese; the other two leaves were taken from manganese-deficient cultures.

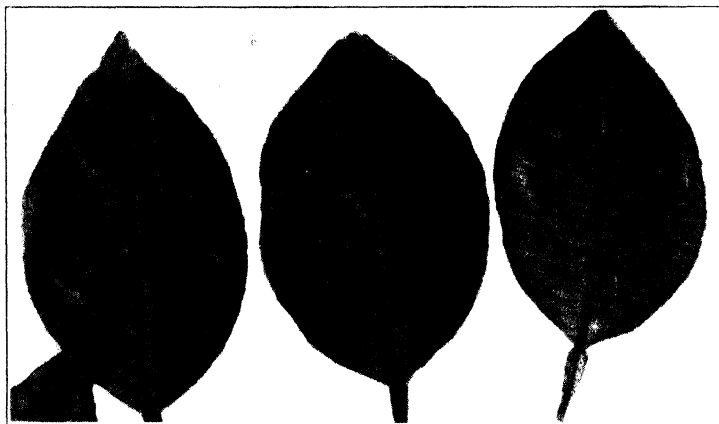
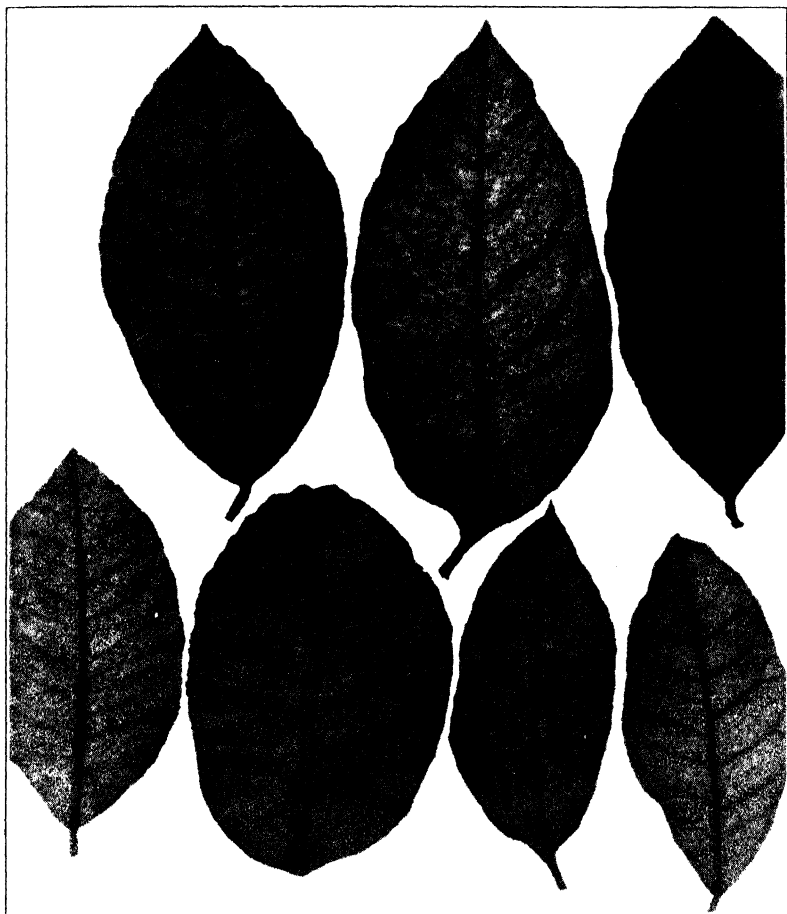


Fig. 2. Young, immature, Valencia-orange leaves from a cutting grown in a culture solution lacking manganese (fig. 14.1). The spotting characteristic of a deficiency of manganese is seen in the leaves of both species.



Rough-lemon leaves from cuttings grown 6 months in culture solutions containing boron, zinc, and purified iron, but no manganese. The leaf on the extreme right in the upper row is from a control culture.



Fig. 1. Typical mature growth of a shoot of a Eureka-lemon cutting grown for 6 months in a culture solution containing boron, zinc, and purified iron, but no manganese.



Fig. 2. Leaves of Valencia-orange trees grown in sand cultures containing finely divided calcium sulfate as the source of calcium and to which the other salts of Hoagland's solution were added, except calcium nitrate. The effects shown in the photograph were produced after a 6 months' omission of manganese and iron.

that attain full size and do not absciss prematurely. In no case do these spots give the leaves a semblance to mottling. Plate 2, figure 1 shows Eureka-lemon leaves taken from cuttings grown 6 months in culture solution containing boron, zinc, and manganese-free iron, but no manganese. The leaf on the extreme right is from a control cutting grown in a

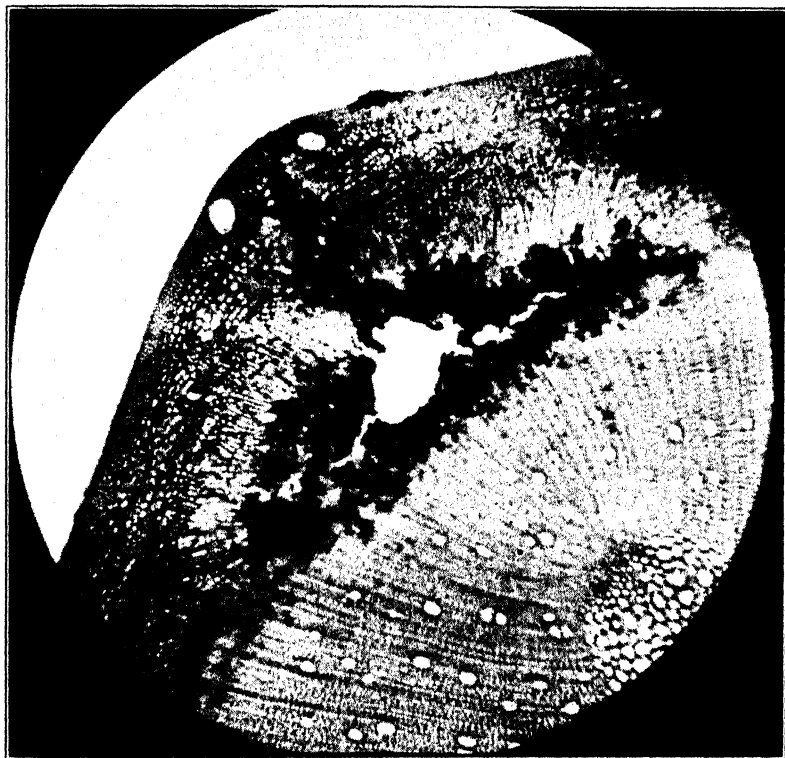


Fig. 10. Cross section of a gum blister on a shoot of a Rough-lemon cutting grown 6 months in a culture solution containing boron, zinc, and purified iron, but no manganese. The section shows the manner in which the gum is exuded from the gum pocket to the surface of the twig.

culture solution containing manganese. The two leaves to the left show the initial stages of manganese deficiency while there was still sufficient manganese available from the previous control culture solution treatment, prior to the present experiment, to enable the leaves to reach full size and maintain a dark-green color. Such leaves may also represent the transitional stage in the resumption of healthy growth when manganese is supplied to cultures suffering acutely from a manganese deficit.

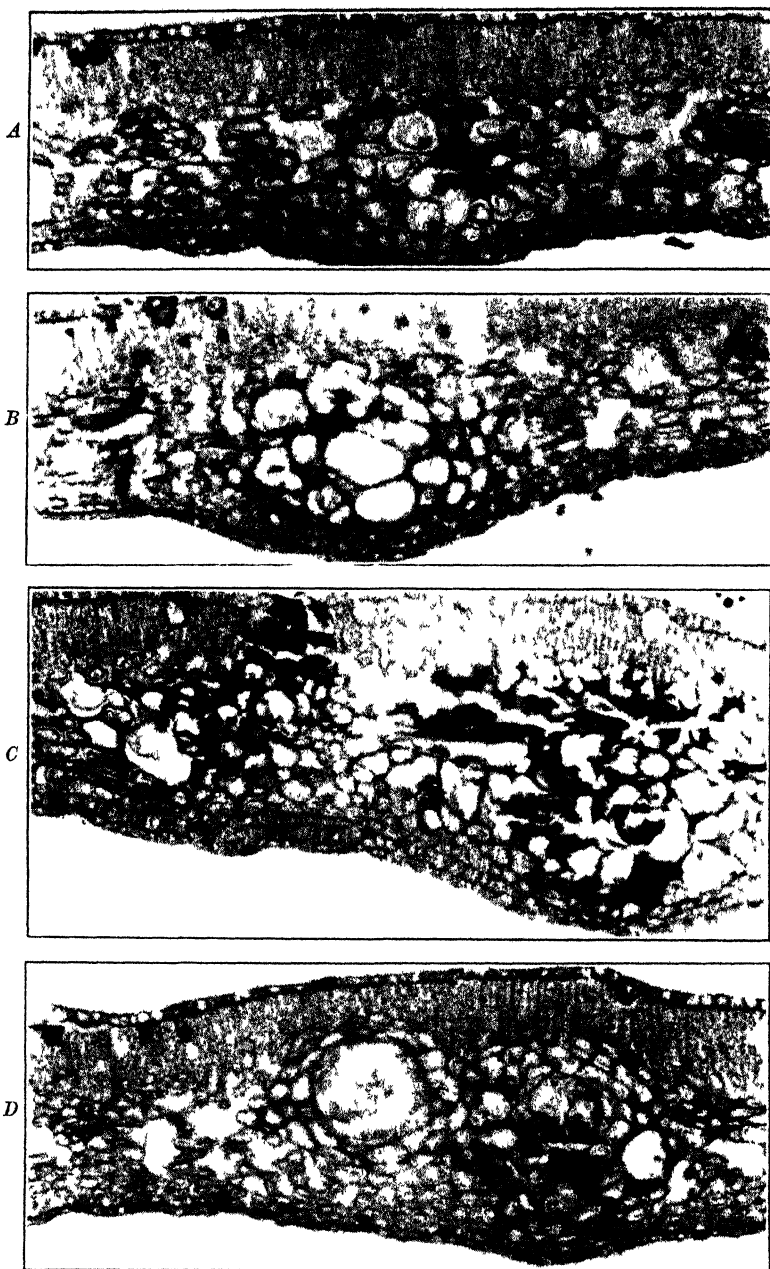


Fig. 11 Vertical sections of lemon leaves from cuttings grown in manganese free culture solutions. *A*, initial stages of cell enlargement and gum formation in the spongy mesophyll; *B*, advanced stage of cell enlargement; *C*, disintegration of cells and advanced stages of gum formation; *D*, gum formation obviously independent of oil glands.

It will be seen that a deficiency of manganese has resulted in the formation of spots which are most dense along the midrib. These spots are small circular or elliptical areas which resemble areas invaded by fungi. They may merge and involve the entire tissue between veins (fig. 11). It is of interest that these spots may become corky or resinous,

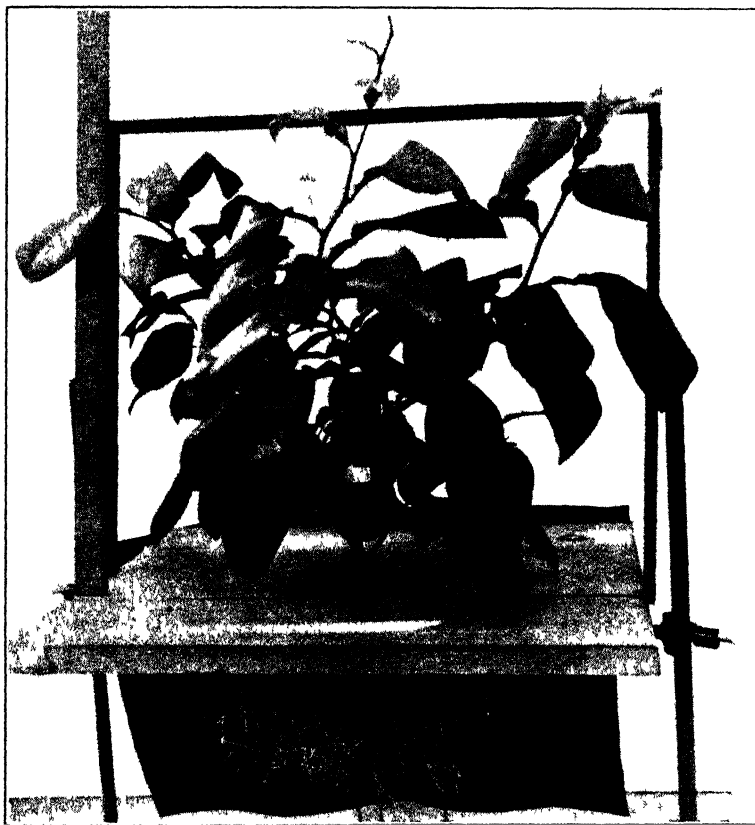


Fig. 12. Sour-orange cutting as a scion on Rough-lemon cutting as a stock grown in culture solution receiving abundant purified iron but no manganese. Compare this with the one shown in figure 13.

or both, on either or both sides of the leaf. Such spots are independent of the oil glands, as may be seen by holding a leaf up to the light, or by making leaf sections. In growing hundreds of lemon cuttings in large pans of culture solution in the past, such spots were often observed but no explanation of their formation was available. They would be present in some leaves and not in others, which now may be explained as due to a shortage in the manganese supply. It occurred largely with

rapidly growing varieties, and the absence of the spotting was associated with the frequency of the iron-tartrate additions in the distilled water, for the more iron tartrate was added, the more manganese was also added as an impurity in the iron supply. Now such spots may be banished at will by simply increasing the manganese supply.

It is of considerable importance that these spots occur in citrus leaves when manganese is deficient, and that they are characteristic of such deficiency not only in the citrus herein reported upon, but they have recently been found by Skinner and Ruprecht<sup>(25)</sup> to be also characteristic of manganese deficiency in the leaves of pepper and other truck crops in Florida. No such spots have been found as yet either in mild or severe cases of mottle-leaf of citrus in the field, and, therefore, it is very unlikely that manganese deficiency is related directly to mottle-leaf of citrus.

In the upper row of plate 3, showing the dorsal surface of Rough-lemon leaves, the two leaves to the left show advanced stages of manganese deficiency. The spots are very numerous, the leaves are a yellowish-green, and resinous spots occur along the midrib. In the lower row is shown the ventral surface of manganese-deficient leaves.

The nature of these spots is of interest. Figure 11 shows vertical sections of Eureka-lemon leaves taken from cuttings affected with a deficiency of manganese. The spots give the leaf surfaces an undulating outline. Oxalate crystals are visible in the upper epidermis of figure 11A. An oil gland is shown in 11D to be independent of the breakdown. The spotting originates from the breakdown of cells first in the spongy mesophyll tissues, especially in the region of very small veins. Other cells become greatly enlarged. The gum formation may proceed through the palisade tissue or through the ventral epidermis. It appears remarkable that such a destructive breakdown of citrus leaves may be prevented by having 0.1 p.p.m. or less of manganese in the culture solution. The results show as conclusively for manganese, as did those of Haas and Klotz<sup>(7)</sup> for boron, the necessity of minute amounts of these elements for healthy growth.

*Effects of Manganese Deficiency on Growth of Orange.*—Sour-orange cuttings were grown as scions on Rough-lemon cuttings in culture solutions. Figure 12 shows one of these trees after a 6-month period of manganese deficiency. Figure 13 shows another of these trees of similar age in the same culture solution but with manganese present. The writer's hope is that such cuttings may be grown to an even larger size, for with an increase in the size of the plant the difficulties of maintaining a nutrition balance in a culture solution becomes more acute and in overcoming the difficulties much information is gained.

Without manganese, Valencia-orange cuttings (fig. 14) grew poorly, even though generously supplied with iron. The leaves were chlorotic and the young leaves were spotted, the immature leaves abscissing in



Fig. 13. Sour-orange cutting as a scion on Rough-lemon cutting as a stock, grown in the same culture solution as that used for the plant shown in figure 12, but with manganese added.

extreme cases. Figure 14A shows some dying shoots from which the leaves have abscised. In some cases where manganese-free iron was added to manganese-free cultures containing cuttings of different species, the roots had a rusty-brown color from the iron supplied, and



yet the leaves were chlorotic. Figure 14B shows the new growth and improved appearance of the cutting shown in figure 14A, 29 days after the first application of manganese. One month later this cutting had new shoots a foot or more in length.

The young leaves of the orange cutting (fig. 14A) grown under conditions of manganese deficiency show a spotting, as may be seen in plate 2, figure 2. Usually the chlorotic spots are more dense near the basal



Fig. 14. Valencia-orange cutting as a scion on sour-orange cutting as stock. A, Condition of culture on July 6, 1931, after 6 months in manganese-free solution; B, condition of culture on August 4, 1931, 29 days after the addition of 5 p.p.m. of manganese as manganese sulfate to the culture solution.

region of the midrib. Sometimes a corking-over of such spots may occur on both surfaces of the leaf but more frequently on the ventral surface. Unless such leaves attain full size, they absciss.

*Manganese and Iron Content of Leaves Grown in Manganese-deficient Solutions.*—When the symptoms of manganese deficiency were well developed, leaf samples were taken just prior to the addition of manganese. The leaves were powdered in a porcelain mortar to prevent possible iron or manganese contamination from a metal grinder. The ash was determined in order to form an opinion regarding the degree of maturity of the leaves. The manganese was determined according to the procedure of Samuel and Piper,<sup>(24)</sup> which is based on the periodate method. The iron was determined by the method of Elvehjem and Hart,<sup>(4)</sup> in which phosphorus was removed and the iron precipitated before estimating the iron colorimetrically with potassium thiocyanate.

Leaves that were produced after the Eureka-lemon cuttings were grown in solution cultures lacking manganese, contained manganese concentrations ranging from 1.3 to 3.3 p.p.m. of dry matter, while the dark-green leaves that were mature on some of these same cuttings before manganese was omitted (control leaves) contained manganese concentrations ranging from 10.7 to 17.0 p.p.m. of dry matter.

It appears that the leaves of the growth brought to maturity after manganese was omitted from the cultures, were unable to absorb sufficient manganese from the leaves that were produced when manganese was not a limiting factor to bring the plane of manganese nutrition in all leaves on each cutting to the same level. This is in agreement with the appearance of the leaves; dark-green leaves grown and matured under conditions of favorable manganese nutrition do not show symptoms of manganese deficiency at any time after manganese has been made a limiting factor. There appears to be a range below which the manganese content of Eureka-lemon leaves cannot be decreased by new growth that is in need of more manganese.

While the lack of manganese has not prevented the absorption of iron in the samples deficient in manganese, the iron content is less (averaging 186 p.p.m.) than in healthy green leaves (averaging 274 p.p.m.) of the same cutting.

If Rough-lemon leaf samples from manganese-deficient cultures are examined, it is found that they consist of old yellow leaves deficient in manganese, their manganese content ranging from 1.6 to 3.2 p.p.m. of dry matter. Rough-lemon leaf samples from control cultures consist of dark-green leaves produced before manganese became a limiting factor, and their manganese content ranges from 5.6 to 8.6 p.p.m. of dry matter. It is of interest to note that a Rough-lemon leaf sample taken from one of the most healthy cuttings grown in solution cultures (see fig. 1) that had always had "A-Y" so as to contain 0.1 p.p.m. of manganese, showed a manganese content of 8.5 p.p.m. in the dry matter and is in good agreement with the results from the other Rough-lemon leaf samples. The Rough-lemon leaf samples deficient in manganese show a lower iron content (averaging 182 p.p.m.) than the control leaf samples (averaging 245 p.p.m.).

The manganese and iron content of the leaves of sour-orange cuttings grown as the scion on Rough-lemon cuttings as stock was determined. One sample consisted of manganese-deficient yellow leaves and the other of dark-green leaves collected from the same cuttings but produced before manganese was made deficient. The leaves of the manganese-deficient cultures contained 1.3 and 141.0 p.p.m. of manganese and iron

respectively in the dry matter, while those of the control sample contained 36.0 and 127.0 p.p.m. respectively. Here the manganese-deficient leaves contained more iron than those leaves that were produced when manganese was not deficient.

When manganese is deficient, in most cases there appears to be less iron accumulated in the leaves. At any rate the leaves have a yellowish cast as though iron were lacking or inactive. In many of these cultures the roots were a rusty brown as a result of the continued supply of

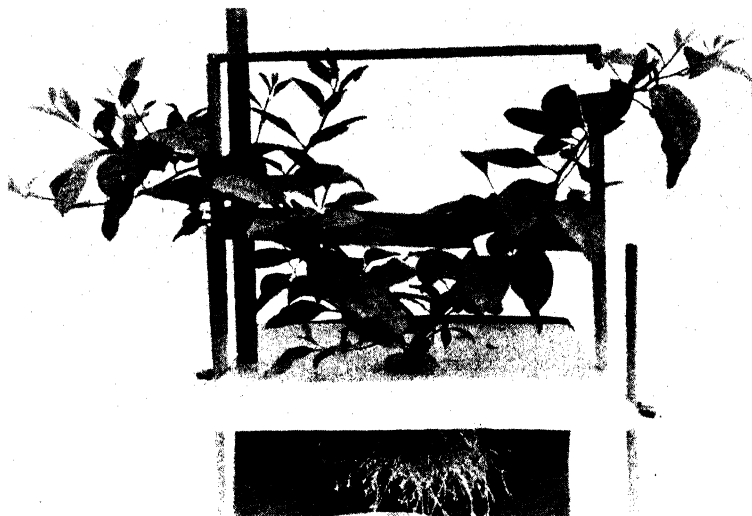


Fig. 15. Rough-lemon cutting grown in a complete nutrient solution for 18 months and then for several months with iron and manganese lacking, which brought about cessation of growth. The addition of manganese initiated vigorous dark-green growth. As the growth matured, the leaves became full sized, but the chlorophyll intensity was gradually reduced until chlorosis was evident.

generous amounts of manganese-free iron. Iron does not appear to function properly when manganese is deficient, but it does not require very much manganese to correct this condition, which may be largely a result of a lack of sufficient oxidizing agent within the cells.

*Symptoms of Iron Deficiency on Rough Lemon.*—The next experiments deal with the lack of iron in the presence of manganese. The plants were grown in a complete nutrient solution for 18 months and then for several months in a nutrient solution lacking both iron and manganese, which caused a cessation of growth. The addition of manganese brought about increased growth within a few days. Figure 15 shows a Rough-lemon cutting in a culture solution containing manganese but no iron. The first young leaves were dark green, owing to

a small reserve of iron within the cutting as a carryover from the control solution containing iron that was used prior to starting the present experiment. As the leaves increased in size, they became more and more yellowish or chlorotic, the apex of each leaf remaining green the longest. In such cultures the omission of iron has caused the leaves to pass through all of the stages of chlorosis to that of albescence. It is of considerable importance to note that the omission of iron, while bringing about chlorosis and albescence, has not caused mottle-leaf.

*Symptoms of Manganese and Iron Deficiencies When Calcium is Supplied as Calcium Sulfate.*—If the manganese and iron are both withheld from sand culture of orange trees that have calcium sulfate as their source of calcium, leaves such as those shown in plate 4, figure 2 may be produced. There is an indication of mottling, and in addition the leaves are covered with numerous yellow spots. Subsequent leaves produced may be chlorotic. The spotting appears to be a result of the decreasing manganese available. The mottling may be a composite result of high-sulfate, low-calcium solubility, and a reduced iron supply; it resembles the mottling in lemon leaves on trees grown in sand cultures supplied with iron and manganese but with calcium sulfate as the source of calcium, whereas the leaves are healthy with calcium nitrate as the source of calcium (Haas and Thomas<sup>(8)</sup>). Orange trees in sand cultures from which iron alone was omitted simply became more yellow, indicating chlorosis. This was confirmed with cuttings in water culture from which iron was omitted. In qualitatively testing the precipitating power of solutions of the various salts used in Hoagland's solution, it was found that not only phosphates but also sulfates rapidly remove iron from an iron nitrate solution.

## SUMMARY

Manganese is necessary for the healthy growth of citrus cuttings in solution cultures.

Although citrus leaves become yellowish green or chlorotic when manganese is deficient, they do not mottle.

Gum or resinous spots occur on either or both sides of the leaves, their number at first being greatest along the base of the midrib. Oil glands in the leaves show no effect from such a deficiency.

Manganese-deficient leaves in acute stages absciss prematurely and the shoots die back. Such shoots may show a resinous excrescence or gum pockets from which gum may be exuded.

The roots remain healthy in appearance even though manganese is deficient for top growth. This may be because the roots have the first

opportunity to absorb manganese and do not surrender any considerable part of it to the leaves during a manganese-deficiency period. The quick response of the growth of new green leaves upon adding small concentrations of manganese to the culture solution may be due to the healthy condition of roots in manganese-deficient cultures.

Excessive concentrations of manganese also bring about chlorosis even though iron is added to the culture solution in similarly large amounts.

Iron is essential for healthy growth in citrus; a deficiency brings about chlorosis. When manganese is deficient in citrus leaves, in most cases less iron appears to be accumulated in the leaves. Mottle-leaf of citrus has not been shown to be a result of iron deficiency. Manganese cannot take the place of iron, and conversely iron cannot take the place of manganese. Most, if not all, compounds of iron contain manganese as an impurity. This fact must be considered in any study of manganese deficiency.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

NOVEMBER, 1932

No. 5

## PLANT BUFFER SYSTEMS IN RELATION TO THE ABSORPTION OF BASES BY PLANTS<sup>1, 2</sup>

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### INTRODUCTION

In view of the important storage and other functions of the parenchyma tissues of agricultural plants, it may be granted that it is of paramount importance that these tissues be kept in a healthy condition. The work of many investigators suggests that a certain degree of constancy of the hydrogen-ion concentration of such tissues is an important factor. To assist in maintaining the proper reaction, a system of buffering in the vacuolar sap with respect to hydrogen ion is presumably necessary. This paper deals with the buffer systems involved as reflected in the sap<sup>4</sup> obtained by expression. The special feature of the investigation was the use of plants grown under controlled conditions of solution or sand-culture technique. Aside from some earlier work conducted in this laboratory, very little study has been made of sap obtained from plants grown in definitely controlled nutrient solutions.

During recent years, Small and his associates have reported the results of many studies on the hydrogen-ion concentrations of plant tissues. A monograph by Small<sup>(18)</sup> contains the data obtained in his

<sup>1</sup> Received for publication February 20, 1932.

<sup>2</sup> In connection with a general investigation, the first part of which is now reported, it is desired to acknowledge the assistance of a grant received from the American Potash and Chemical Company.

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<sup>4</sup> Various terms are used to designate the fluids expressed from plant tissues: sap, tissue fluids, plant juice, etc. Objections can be made to any term employed. In this paper the common term "sap" is used for convenience.



laboratory, by himself and his coworkers, Martin, Ingold, and Armstrong. Much work has been done on individual tissues, the pH values being obtained colorimetrically by what is termed the "range indicator method" or "R.I.M." A discussion of this work is beyond the scope of this paper. In the consideration of buffer systems, Small and his associates have confined their attention especially to a limited range of pH between 4 and 7. This is doubtless because the pH of the sap of most agricultural plants lies within this range and because changes around the actual pH of the plant may be assumed to be the only ones of importance from the point of view of the preservation of a suitable hydrogen-ion concentration.

In undertaking the work to be presented herein, it was felt that only by a study of the complete buffer system could the buffering mechanism at any point be fully elucidated. The evidence obtained indicates that this supposition was correct. Furthermore, the titration of expressed plant saps to low and high pH values has a distinct interest, apart from the question of pH or of buffering. Reflections are thus obtained of variations in concentration of important metabolic constituents of the plant. The primary purpose of this paper is to present evidence on the relation of plants to their nutrient medium, as reflected by the composition and buffer of the sap expressed from the tissues with a given technique.

## TECHNIQUE

The following investigations have all been conducted on the expressed sap of plants. While the contents of the cells of many tissues are represented in the composite sap so obtained, it is certain that the bulk of it comes from parenchymatous tissue. Although it is unquestionably necessary to emphasize and to make allowance for the uncertain and composite character of saps expressed from plant tissues, the important point now is that consistent reflections of metabolic conditions may be obtained by the study of expressed saps under suitably controlled conditions. The ideal of quantitative study of each type of cell or tissue is not yet attainable. Small<sup>(18)</sup> objects that the pH values of expressed saps are of limited validity owing to the loss of CO<sub>2</sub> when the sap comes in contact with air. Further discussion will try to show that this objection is not so important as it might appear at first sight, at least for the objectives of the present investigation.

The principal methods of obtaining sap from plant tissues are: (1) grinding the material to a pulp and extracting by pressure; (2) injur-

ing the cells with organic substances, such as ether, and then obtaining the sap by pressure; (3) freezing, thawing, and pressing plants.

The first method gives juice containing suspended matter, and is hard to filter. The second and third were compared by Copeland<sup>(1)</sup> of this laboratory, who found that the sap obtained by these methods was similar in character.

The method of freezing and thawing was adopted in the present studies. The plant tissues were placed in closed bottles as soon as harvested, and then immediately set in a freezing chamber, kept at about  $-15^{\circ}$  C. The tissues were later thawed at room temperature, and pressure was applied while the material was still cold.

The screw press consists of a heavy steel casing, into which is first inserted a short cylinder perforated with small holes. The material to be pressed is enclosed in cloth and placed above this cylinder. Another close-fitting steel cylinder about 4 inches in diameter and 8 inches in height is then set in place. Pressure is applied by a shaft threaded through a steel bridge. This shaft is fitted to a 16-inch diameter wheel, which is turned by hand. The sap runs through the lower perforated cylinder and comes out through an opening on the side of the casing. In most cases it is filtered rapidly through filter paper and titrated at once. In some of the earlier experiments it was allowed to stand overnight in a cool place. For present purposes, the use of extremely high pressures was considered unnecessary, and in fact, undesirable. The intention was to secure a sap as nearly as possible approximating the vacuolar sap. The complete disintegration of tissue was not sought.

All measurements were made with the Bunker type of hydrogen electrode. At first the electrode was platinized for 10 seconds after each titration and hydrogen gas was secured by electrolysis of NaOH solution. Later it was found unnecessary to replatinize the electrode so frequently, provided it was dipped in dilute acid and then thoroughly washed with water after each alkali titration. Recently it became more convenient to use hydrogen gas from a cylinder. In both cases the hydrogen gas was passed over heated platinum black in order to remove oxygen. Measurements were made on a Leeds and Northrup potentiometer reading pH directly. The instrument is guaranteed to be accurate to 0.01 pH. More accurate instruments are sometimes employed in experiments on plants, but the inherent biological errors make it very doubtful whether anything is to be gained by further refinement of the physical-chemical technique. The temperature at which all measurements were made was approximately  $25^{\circ}$  C.

Some experiments were conducted to determine the accuracy of the technique used. Readings of pH taken on a buffer solution of  $\text{KH}_2\text{PO}_4$  showed a maximum deviation of 0.005 pH from the mean, and readings were recorded to nearest 0.01 pH.

Five 50-gram samples of the tops of wheat plants were taken from a large lot and pressed out separately after freezing. The values showed maximum deviations of 0.03 pH on either side of the mean. As a result of this experiment, differences of pH are not ordinarily considered significant if less than 0.1 pH.

The pressure needed to obtain a representative sample of sap was determined. The first half of the sap could be secured with very little pressure—the second half required the full power of the press. Titration showed that practically identical pH values and buffers were obtained in each half.

The importance of freezing before pressing was shown by the fact that while 5 cc of sap expressed from unfrozen wheat plants required only 2.66 cc of acid and alkali for the buffer over the range of pH 2.0 to 10.5, the same amount of sap from the frozen plants required 6.40 cc to cover the same range. There was also a difference of 0.3 in the initial pH of the sap.

In one experiment, a lot of wheat plants was divided into five portions which were separately frozen and thawed, then successively pressed; pH determinations were made immediately after pressing. The readings were as follows:

1st sample.....	pH 5.87
2nd sample.....	pH 5.89
3rd sample.....	pH 5.92
4th sample.....	pH 5.91
5th sample.....	pH 5.93

There appears to have been a rise in pH on standing before pressing, but of less than 0.1 pH. At the same time the effect of standing after pressing was specifically investigated. The pH of a sample of fresh sap was found to be 5.83, and after standing overnight in a cool place it was 5.77. Similar comparisons were made on other samples. The changes were observed to be within the 0.1 pH limit of error noted above.

An experiment was then planned to determine more definitely whether allowing the tissues to stand at room temperature after thawing, but before pressing, had any effect. It was also planned to determine whether standing after cutting, but before freezing, had any effect on the pH of the sap. A collection of buckwheat leaves was made and divided into five portions. Four of these were frozen immediately and

the other was left standing in a closed container in the greenhouse for three hours before freezing. The results are given in table 1.

TABLE 1  
EFFECT OF ALLOWING LEAVES TO STAND AFTER HARVESTING BUT BEFORE FREEZING,  
AND AFTER THAWING BUT BEFORE PRESSING, AND OF SHAKING ELECTRODE  
WHILE MAKING READING, ON THE pH VALUE OF THE SAP

Sample No.	Treatment	pH value of the sap	
		Without shaking electrode	Shaking electrode while making reading
1	Stood 3 hours before freezing; pressed as soon as thawed .....	5.35	5.35
2	Frozen at once; pressed as soon as thawed .....	5.34	5.34
3	Frozen at once; pressed 20 minutes after thawing .....	5.46	5.46
4	Frozen at once; pressed 3 hours after thawing .....	5.52	5.45
5	Frozen at once; pressed 3 hours after thawing .....	5.52	5.45

In the case of sap from plants which stood after thawing, a lower pH value was obtained when the solution was shaken while the electrometric reading was being made. This suggested that some substance was reduced by  $H_2$  at the surface of the electrodes, possibly with the formation of ammonia. Such an effect was not observed with sap from samples pressed immediately after thawing.

In the absence of experimental data any explanation of these observations is speculative. Nightingale, Schermerhorn, and Robbins<sup>(17)</sup> report an increase in the amino acid content of sweet-potato roots when these were allowed to stand subsequent to thawing. Lincoln and Mulay<sup>(11)</sup> found that after 24 hours' standing, hydrolysis of proteins had occurred in the bark of pear trees. It is possible that changes of this character might be of greater magnitude in the leaves of plants, and that they are responsible for the observed slight alterations of pH.

After a number of samples of sap have been prepared for titration, it has been the practice to titrate all with alkali and then later all with acid. The acid titration is usually done about 2 hours after the alkali titration. It has been observed that frequently the initial pH taken on the second sample of sap (used for the acid titration) is slightly lower than that of the first. The number of cases in which this has occurred is so great as to make it evident that on standing the  $H$ -ion concentration of the sap is often increased. The change is usually less than 0.1 pH, and is probably a result of the formation of organic acids. Considerably longer standing does not seem to produce a further measurable change. Another possible source of error is the condensation of moisture on the cold tissues before pressing. However, such dilution would not appre-

ciably alter the trend of the buffer curves and probably would not change the initial pH beyond limits of other errors. In any given experiment, all sets of plants were treated alike as far as was possible.

## THE PLANT BUFFERS

There are many substances which might be responsible for the buffering effect in plant sap. The evidence concerning the more important ones will be given consideration. A survey of the literature suggests the following substances:

1. Soluble protein material
2. Carbonates
3. Phosphates
4. Salts of organic acids
5. Amino acids and their amides

*Soluble Protein.*—The amphoteric nature of protein material around its isoelectric point suggests that it may be of importance in the living plant, in the maintenance of a definite hydrogen-ion concentration. The experiments of Hurd-Karrer,<sup>(4)</sup> Martin,<sup>(13)</sup> and Youden and Denny<sup>(21)</sup> indicate that actually proteins are not of importance in the buffer system of the sap.

*Carbonates.*—Carbonates are of importance in the buffer metabolism of blood, and must be considered as possible constituents of plant buffers. Copeland,<sup>(11)</sup> working in this laboratory, was unable to detect appreciable amounts of carbonates in the sap expressed from young pea plants. Martin<sup>(14)</sup> reports CO<sub>2</sub> present in sunflower sap, but in amounts probably too small to constitute an important part of the buffer system. Leuthardt<sup>(9)</sup> considers the amount of carbonates in fruits and succulent plants to be unimportant.

Small<sup>(18)</sup> lays considerable stress on the CO<sub>2</sub> found in sap. He objects to the use of hydrogen electrode determinations on expressed sap on this basis. He maintains, correctly enough, that when the expressed sap comes in contact with the air, any excess CO<sub>2</sub> will be lost. Furthermore, in the act of saturating the solution with hydrogen, the remaining CO<sub>2</sub> will be lost. However, it is very doubtful whether much CO<sub>2</sub> will be found in the sap from leaves or stems of agricultural plants with the technique usually employed. The plants are generally harvested during a period of illumination when the CO<sub>2</sub> available is being used in photosynthesis. Martin<sup>(14)</sup> reports 7 per cent CO<sub>2</sub> in the broad bean (*Vicia faba*), but most plants are buffered strongly enough so that the shift in pH caused by such a concentration would be very small.

It may be suggested, therefore, that when the tops of plants are harvested as in the present investigation,  $\text{CO}_2$  is of minor importance, either as a determinant of the buffer system or of the actual pH of the sap, at least of the composite sap. The effect of  $\text{CO}_2$  in certain specialized cells may fail to be reflected in such sap. Moreover, much of the value of the experiments to be described herein is found in the comparisons of saps obtained by a standard technique, when plants are grown under diverse and known cultural conditions.

*Phosphates.*—Phosphates have an important rôle in the buffer system of the blood. In addition to buffering between pH 4.5 and 7.0, they have a buffer action around pH 2 and pH 13.

The amounts of phosphate usually found in plant saps are fairly small but around the neutral point may be very important. Martin<sup>(12, 13)</sup> found that between pH 6 and 7, the portion of the buffer ascribed to phosphate varied from 100 per cent in the case of sunflower, to 50 per cent in broad bean. According to Ingold,<sup>(7)</sup> phosphates account for only 35 per cent of the buffer between pH 6 and 7 in the potato tuber. It is certain that if phosphates are present, they will have some buffer action. The varying degrees of importance to be assigned to phosphate as a buffer will depend on the amount present, and on the presence or absence of some other substance effective over the same range of pH.

*Salts of Organic Acids.*—Organic-acid radicals are well-known constituents of plant sap, and earlier, as well as recent investigations, make it evident that such acids as citric, malic, oxalic, tartaric, etc., together with their salts, are of great importance in the plant buffer system.

*Amino Acids and Amides.*—These substances have received very little attention as being of possible importance in the buffer system. Ingold<sup>(7)</sup> found that a 3 per cent solution of asparagine had very little buffer between pH 6 and 7. Youden and Denny<sup>(21)</sup> used a solution of glycocol, comparable with the amino acid nitrogen present in potato extract, and observed very little buffer. Leuthardt<sup>(9)</sup> believed that glutaminic acid is responsible for the buffer of mesembryanthemenum on the alkaline side of neutrality, but that amides are not important around the actual pH of plant saps. Vickery<sup>(19)</sup> has found appreciable quantities of aspartic and glutaminic acids in alfalfa sap. Asparagine is well known to occur in many plants.

*Other Substances.*—Sugars exhibit a buffer effect above pH 9, but a fairly concentrated solution is necessary. While such concentrations are present in some fruits, they are not usually found in the green tissues of agricultural plants.

## DUPLICATION OF BUFFER CURVES WITH ARTIFICIAL SOLUTIONS

In 1929,<sup>(2)</sup> the writer attempted to duplicate the buffer curves of saps from buckwheat stems by an artificial mixture. A resumé of the work (unpublished) will be given here.

It was realized that if proteins were important the system would be extremely complex. Experiments were conducted to see if the proteins could be eliminated from consideration. Boiled and filtered sap was compared by means of buffer titrations with fresh sap, and the curves were found to be identical. In another case wheat plants were divided into two lots, the one being dried and ground, and the other frozen. Water was added to the dried and ground sample to give the same water content as fresh plants, and an extract obtained by the use of pressure. The acid buffer curve of this extract was compared with that of the expressed sap of the frozen plants, and they were found to agree almost exactly.

To separate proteins, the expressed sap of fresh tissues was dialyzed for 60 hours and then titrated. The dialyzed sap had a lower pH and a little more buffer than the fresh sap; but no attempt had been made to inhibit enzyme action, and it is believed that this was responsible for the change in pH. A sample of undialyzed sap which stood for 60 hours had the same pH value as the dialyzed sample. It is fairly certain that the buffering substances can be dialyzed and that proteins are of no great importance, as far as the sap itself is concerned. Obviously the buffering system within the protoplasm cannot be disclosed by experiments of the type reported in this paper.

An attempt was then made to duplicate buffer curves of the expressed sap from the stems of two sets of buckwheat plants. These plants had been grown under controlled culture solution conditions, one solution being fairly high in K and the other low. Slight differences in the curves reflecting the two treatments were noted (fig. 1). An attempt was first made to duplicate the high-K curve, since this was considered to be representative of normal buckwheat stems. A complete inorganic analysis of the sap had been made, and phosphate was used in the concentration determined. By mixing the phosphate with suitable organic acids, the curve for the sap could be duplicated on the acid side of pH 7, but in order to obtain a buffer on the alkaline side, the addition of amino acid and amide was found necessary. The inorganic analysis had shown that most of the cation content was made up by K and most of the

inorganic anion content by  $\text{NO}_3$ . The total equivalents of all cations and of all inorganic anions, except phosphate, were then calculated and equivalent amounts of KOH and  $\text{HNO}_3$  added. The following mixture was found to give a curve approximating fairly closely the high-K curve:

Asparagine .....	0.012 M
Aspartic acid.....	.021 M
Malic acid.....	.020 M
Phosphoric acid.....	.015 M
KOH .....	.160 N
$\text{HNO}_3$ .....	0.100 N

The initial pH of the mixture was 4.32.

An attempt was then made to duplicate the low-K curve using different quantities of the same constituents. The amide and amino acid were increased to give the increased alkaline buffer observed. KOH,  $\text{HNO}_3$ , and phosphate were added in accordance with the indications of the sap analysis. As the initial pH of both high-K and low-K saps had been the same, it was found necessary to lower the organic-acid content to bring the solution to the pH of the original sap. The constituents of the solution were as follows:

Asparagine .....	0.016 M
Aspartic acid.....	.026 M
Malic acid.....	.004 M
Phosphoric acid.....	.015 M
KOH .....	.140 N
$\text{HNO}_3$ .....	0.100 N

The initial pH of the solution was 4.22. The buffer curves obtained from the high and low-K mixtures were then compared with the original curves and with each other. They are shown in figure 1.

It was evident that insufficient buffer had been obtained on the acid side of pH 7 in the low-K artificial mixture (fig. 1D). It is possible that this could have been corrected by a partial replacement of malic acid by citric acid, which buffers at the required pH. However, the work was suspended at this point, since its purpose was to indicate the classes of substances responsible for the buffer of the sap, rather than the actual substances. It was shown that amino acids and their amides, which had previously not been considered of importance, might play a large part in the buffering effect over the range studied.

Similar results have recently been published by Hurd-Karrer,<sup>(5)</sup> using mixtures of phosphate, asparagine, leucin, malate, and glucose. She was able to duplicate the buffer curves obtained for the sap of wheat seedlings. The amount of glucose used was about three times as much



as was actually found in the sap. Hurd-Karrer considered that the excess may represent other soluble carbohydrates or other substances buffering above pH 9.5.

As has been emphasized by Hurd-Karrer<sup>(13)</sup> and by the writer,<sup>(2)</sup> it is possible that the same buffer curves could be obtained using an entirely different group of substances. That the curves could be duplicated by other substances known to occur in plant sap is not so likely.

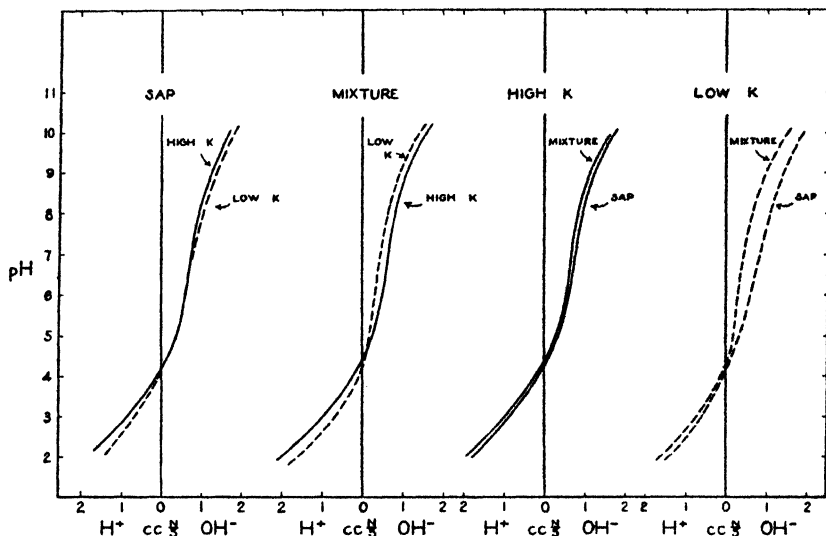


Fig. 1. Comparison of buffer curves of artificial mixtures containing different amounts of K, with each other and with curves of buckwheat stem sap, high and low in K; 5 cc sap was used in titration.

As Hurd-Karrer has pointed out, "it does not seem probable that the close agreement between the titration values of the buffer mixtures and those of the different juice samples is entirely fortuitous." In spite of this, a complete analysis of the sap is necessary before anything can be regarded as proved.

It seems reasonably certain that the buffer on the acid side is mainly due to organic-acid radicals. The alkaline side is more problematical. Analyses of buckwheat sap were therefore made to determine whether aspartic acid and asparagine, when substituted mol for mol for the amino acid nitrogen and the amide nitrogen found in the sap, would duplicate the buffer on the alkaline side of neutrality. The amino nitrogen was determined by use of the Van Slyke apparatus. For amide nitrogen the sap was digested with HCl, made alkaline with MgO, and the ammonia distilled over into  $\text{H}_2\text{SO}_4$ . Recent experiments by Vickery and

Pucher<sup>(20)</sup> indicate that  $\text{H}_2\text{SO}_4$  rather than  $\text{HCl}$  should be used in hydrolysis for amides, otherwise low values may be obtained. The amino nitrogen was found to be 0.0077 M and the amide nitrogen to be 0.004 M. The methods available for determining the organic acids were unsatisfactory, but an estimate of 0.03 M was made for malic acid. Phosphate was found to be low, only 100 p.p.m. being present. For purposes of titration, this was regarded as 0.001 M.

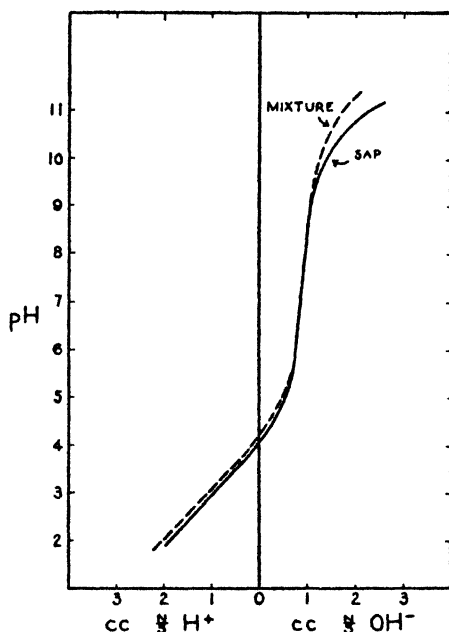


Fig. 2. Comparison of buffer curves of artificial mixture and of sap from buckwheat stems; 5 cc sap was used in titration.

These substances were then mixed to the above concentrations and a titration curve made. This was compared with the curve of the original buckwheat sap. There was not enough buffer above pH 9. Glucose was therefore added to give  $\frac{\text{M}}{2}$  concentration, but even then the original curve was not duplicated. While fairly close agreement (fig. 2) was obtained on the acid side, the curves separated above pH 10. The artificial mixture used was as follows:

Aspartic acid .....	0.008 M
Asparagine .....	.004 M
Malic acid.....	.03 M
Oxalic acid.....	.01 M
Phosphoric acid.....	.001 M
Glucose .....	0.5 M (7 per cent)

To this mixture NaOH was added to bring it to the pH of the original sap. The mixture was then titrated with HCl and NaOH as in the case of the sap.

The results clearly show a discrepancy on the alkaline side, particularly as it is extremely unlikely that 7 per cent glucose would be found in buckwheat sap. However, if leucin had been used as by Hurd-Karrer, or perhaps a mixture of leucin and aspartic acid, better agreement might have been obtained. The alkaline pK value for aspartic acid is 12.1, while for leucin it is 9.8. This latter should provide more buffer over the pH range where the discrepancy is most marked. The asparagine added seems to supply enough buffer over the part of the curve represented by that substance.<sup>5</sup>

This experiment suggests further that the classes of substances indicated independently by Hurd-Karrer and by the writer as being responsible for the buffering effect in many types of agricultural plants are probably the correct ones.

While the evidence presented earlier indicates that the buffer effect of one type of substance will merge into that of another type, the buffering range of each is sufficiently definite to give some information concerning changes which have taken place. At least, if two sets of plants grown under different conditions should give the same pH value and identical buffer curves over a sufficient range, it is likely that the principal organic constituents of the sap would not differ to any great extent. An attempt was made to estimate approximately the amino acid content of a sap from titration data, but owing to the overlapping of curves for amides, sugars, and amino acids, this was found to be impractical. However, it is believed that a qualitative idea of the concentrations of amino acids, amides, and total organic acids can be obtained by inspection of buffer curves. Small<sup>(18)</sup> has shown that it is often enlightening to compute "buffer indices" from the titration curves. In connection with the present discussion, it has not seemed essential to present the data in this form, since the titration curves clearly show the influence of the culture media on the buffer system of the sap. The general classes of substances involved in the buffer system are indicated by inspection of the curves and from other data. The amount of sap used in all titrations was 5 cc, and from the data presented, "buffer indices" can be computed if desired.

<sup>5</sup> It has been suggested that phenols and catechols may possibly have a buffer effect in the alkaline range.

## INFLUENCE OF ILLUMINATION OF PLANT ON pH OF SAP

Diurnal changes in the pH of sap from succulent plants are known to occur. Such changes are often of large magnitude. The evidence for similar variations in agricultural plants has been recently reviewed by Loehwing.<sup>(10)</sup> Perhaps the largest changes are those cited by Ingalls and Shive.<sup>(6)</sup> They report that buckwheat stem sap may vary from pH 4.4 to 4.8 and leaf sap from pH 4.9 to 5.4, according to the time of day. Accumulation of organic acids during the night and photolysis during the day is believed to be responsible for these changes. Such large differences had not been observed in this investigation. An attempt was therefore made to accentuate the effects of light and darkness.

Buckwheat plants growing in a culture solution were selected and divided into three sets. Set 1 was harvested in the afternoon and at the same time set 2 was placed in darkness. On the following day, set 2 was harvested after 24 hours in darkness, and at the same time set 3, which had been in light all day, was also cut. These tissues were all frozen immediately after harvesting and subsequently used for pH determinations.

The buckwheat plants were pressed out immediately on thawing, with the following results:

	pH
Set 1.....	5.28
Set 2.....	5.21
Set 3.....	5.27

In a similar experiment with tomato plants which were let stand some time at room temperature before pressing, the following values were obtained:

	pH of stem	pH of leaves
Set 1.....	5.66	5.73
Set 2.....	5.61	5.65
Set 3.....	5.68	5.80

The changes of pH are very small and may not be significant.

It must also be concluded, from the work of Loehwing<sup>(10)</sup> that while changes in pH may be produced by the effect of light, they are not always of the large magnitude reported by Ingalls and Shive. Data thus far published do not permit comparisons of temperature effects during the dark periods. It is to be kept in mind that in the present investigation, plants from any one experiment were harvested as nearly as possible at the same time of day.

A few experiments were made to ascertain whether the pH of the expressed sap could be altered by treating the plants with the rays from a mercury arc lamp (5 minutes' daily exposure for 2 weeks). No significant change in pH was found in these particular experiments.

## INORGANIC NUTRITION OF THE PLANT IN RELATION TO BUFFER SYSTEMS

Apart from the effect of calcium, little investigation has been made of changes in the buffer system of the sap induced by modifications in the inorganic nutrition of the plant. It is, of course, logical to endeavor to explain the buffer components before attempting to identify variations in the curves. Some of the work reported below was done in the hope that it might throw some further light on the constituents of the buffer system. In the main, however, it was carried out when some knowledge of the system had already been obtained, as described in the preceding sections of the paper.

*Phosphorus.*—Martin<sup>(14)</sup> has shown that in bean plants, over a narrow range of pH, the concentration of phosphate in the plant sap may account for all the buffer. That this is not always the case was demonstrated by Ingold,<sup>(7)</sup> who found that in potato tuber the phosphate could account for only about 30 per cent of the buffer over the same range of pH.

In view of the marked effects of subjecting plants to a low supply of phosphate, it was considered probable that the cell sap might show some change as a result of the evidently deranged metabolism. Water-culture experiments with wheat plants were carried out. The phosphate supply in the low-phosphate set was decreased enough to produce a marked decrease of yield. Except for decreased yield, these plants appeared normal. The sap was obtained and titrated.

It is evident that there was a small decrease of pH resulting from the low phosphate supply. There was also a considerable increase in the buffer on the alkaline side, which probably indicates an increase in amides, amino acids, or sugars (fig. 3). Kraybill<sup>(8)</sup> has reported analytical data showing an increase of amide and amino nitrogen in plants grown under conditions of low phosphate supply, and similar results have been obtained in this laboratory.

*Calcium.*—Various earlier investigations have emphasized the assumed necessity for Ca or  $\text{CaCO}_3$  for neutralization of organic acids produced in the course of plant metabolism. The effects of liming soils

on the reaction of plant sap have received much attention. Frequently the results of such experiments are lacking in consistency, and the biological errors involved have not always been given due consideration.

Loehwing<sup>(10)</sup> has recently studied wheat plants grown on humus and loam soils. The plants from the lime-treated soils in all cases showed a decrease in acidity. There was a larger change in pH in the plants from the humus soil treated with lime than in those from the loam soil,

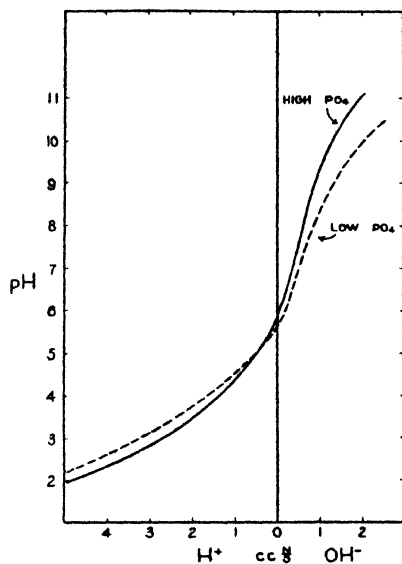


Fig. 3

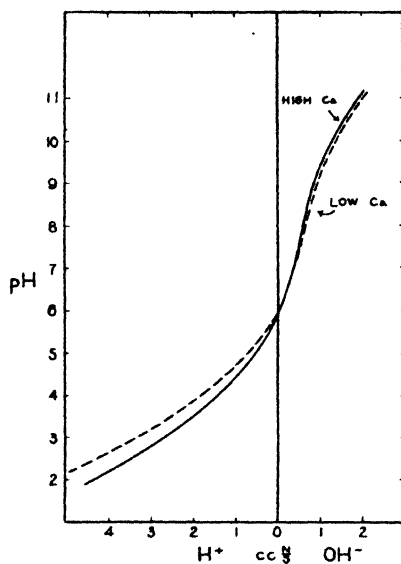


Fig. 4

Figs. 3 and 4. Buffer curves for tops of wheat plants (Little Club) grown in solutions indicated in each chart; 5 cc of sap was used for titration.

The solutions were of the type described in table 4. In the low- $\text{PO}_4$  solution,  $\text{KH}_2\text{PO}_4$  was used in 0.0001 M concentration.

The plants were grown in a greenhouse approximately 6 weeks from February 24, 1928. Two-liter jars were employed, with two plants in each jar.

but the former showed signs of chlorosis. Loehwing considered that the acidity developed in plants grown on the untreated humus soil and the alkalinity developed by large additions of lime were both injurious. The most vigorous plants were grown on the loam soil, and the differences of pH between plants grown on the limed and unlimed soil, while definite, were smaller than on the humus soil, being usually about 0.2 to 0.3 pH.

Dustman<sup>(8)</sup> used tomato plants grown in water cultures to investigate the effect of Ca. The plants were grown at pH values of 4, 5, and 6, concentrations of Ca being 1,000, 100, and 10 p.p.m. While the low-Ca

plants showed small increases in acidity of sap, it was believed that in view of the variations found in duplicate samples, the differences were not significant. Newton<sup>(15)</sup> also grew pea plants in solutions of high and low Ca content and found no increase in acidity associated with low Ca supply.

It seems probable that the internal pH of plants may sometimes be definitely changed, usually within a narrow range, by large applications of  $\text{CaCO}_3$ , but it has not been adequately proved that such applications are actually indispensable for the purpose of preventing an injurious lowering of the pH of the sap. To investigate this point further, plants were grown under conditions of high and low Ca supply. In order to control the supply of Ca accurately, culture solutions were used. Wheat plants were grown in solutions containing 4 p.p.m. and 100 p.p.m. of Ca. In all solutions in which the Ca concentration was decreased, the Mg concentration was also decreased with the idea of avoiding any possible complications in the relation of Ca to Mg. Determinations of pH and of buffer were made in the usual way on the expressed sap. No significant difference of initial pH was observed, but the low-Ca treatment produced an increase in the buffer against acid (fig. 4). Analyses for all the inorganic cations and anions were made.

Table 2 shows that a significant lowering of the Ca content of the sap resulted from the low-Ca treatment. The cations and anions were calculated as milliequivalents per liter, and the total of equivalents of cations compared with the total of anions as determined. Assuming that the excess cations were in equilibrium with organic-acid radicals, it is seen that, in spite of the lowering of Ca in the culture solution, and also in the sap, more bases are found in combination with organic-acid radicals in the low-Ca sap than in the high. The increase in buffer shown by the titration curve is consistent with this finding.

A different condition with respect to Ca exists in buckwheat sap. It has been assumed by other investigators that in this plant Ca is necessary to precipitate oxalic acid, which would otherwise be injurious by reason of its toxic nature. While oxalic acid may be injurious to animals, as far as the writer is aware it has not been proved that the oxalate ion, when divorced from the actual acidity produced by oxalic acid, has any toxic effect on plant growth. However, it is true that deposits of calcium oxalate are found in many plants, including buckwheat, and for this reason, oxalates are of special interest.

Buckwheat plants were grown in culture solutions containing 4 p.p.m. and 100 p.p.m. of calcium. Similar growth was made under each condition, although the yield from the low-Ca solution was about

20 per cent greater. At harvesting, the leaves and stems (including petioles) were separated, and buffer curves were obtained on the expressed saps (fig. 5). There was no change of initial pH for either the

TABLE 2

ANION AND CATION CONTENT OF SAP OF WHEAT PLANTS GROWN UNDER LOW AND HIGH-Ca CONDITIONS

Culture solution	Ca, in p.p.m.	Total cations	Total inorganic anions	Excess cations
		Milliequivalents per liter		
Low Ca .....	75	162	19	143
High Ca .....	330	197	64	113

stems or leaves, but as in the case of the wheat, the buckwheat plants had a greater acid buffer in the low-Ca solution than in the high. The sap from the stems will be considered more fully. Table 3 gives a summary of the analytical results. Owing to the fact that most of the Ca in

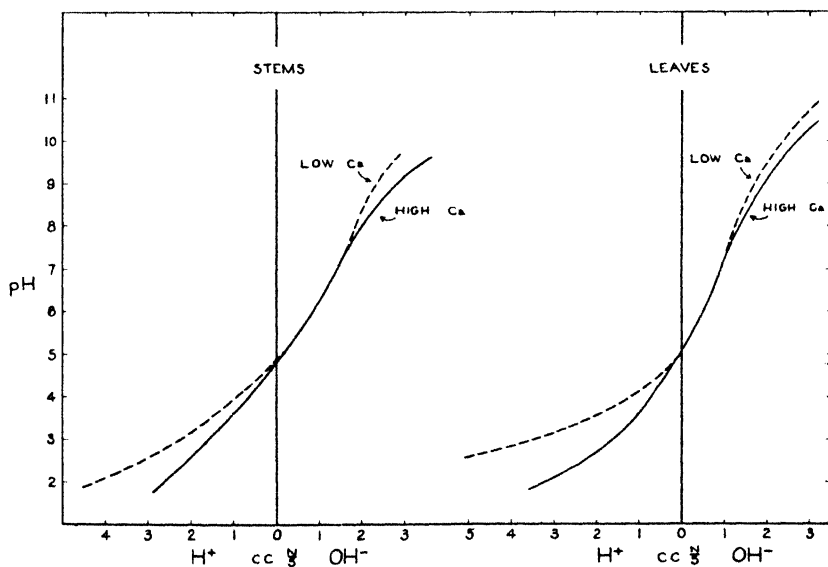


Fig. 5. Buffer curves of sap from buckwheat stems and leaves; plants grown in high and low-Ca solutions. Five cc sap was used in titrations.

buckwheat is insoluble, the concentrations are low in the sap from plants of both sets. The Ca contents of the residues left after expressing the sap were 0.20 per cent and 1.43 per cent on the dry basis, for



the low-Ca and the high-Ca plants respectively, thus proving that the low-Ca treatment was effective in reducing the calcium content of the plant as a whole. Both total equivalents of cations, and the excess of equivalents of cations over inorganic anions, were greater in the low-Ca plants (table 3). More base was available for combination with organic acids in the low-Ca plants than in the high-Ca plants, and the titration curves are consistent with this fact. The same relations were observed with the buckwheat leaves.

The decrease in Ca content of the sap was accompanied by a large increase in K. The latter then served as the main base in equilibrium with organic acids, and it is suggested that in the buffer system of the sap one base serves as well as another, provided enough total base can be absorbed. As K is readily absorbed by most plants, it is possible that the absence of Ca from the buffer system of plants of the type under discussion may have no ill effects, provided that it is present in sufficient amounts for other purposes of plant metabolism.

The changes in pH of saps as recorded in the literature have been mainly produced by applications of lime to soils. A sand-culture experiment was planned to investigate this phase of the problem. Two-gallon crocks of pure white sand were prepared and to each crock was added 1,500 cc of culture solution containing 100 p.p.m. Ca for the high-Ca set and 20 p.p.m. for the low-Ca set. In the third set  $\text{CaCO}_3$  was mixed

TABLE 3

ANION AND CATION CONTENT OF SAP FROM BUCKWHEAT STEMS GROWN IN LOW AND HIGH-Ca CULTURE SOLUTIONS

Culture solution	Ca, in p.p.m.	Total cations	Total inorganic anions	Excess cations
		Milliequivalents per liter		
Low Ca .....	59	317	182	135
High Ca .....	123	193	92	101

with the sand to give a content of 1.5 per cent  $\text{CaCO}_3$ , on the basis of dry sand. Five buckwheat plants were grown in each crock. The growth was about the same with high and low Ca supply, but the  $\text{CaCO}_3$ -treated plants became chlorotic. Iron tartrate was added frequently in an attempt to correct this condition, but the ultimate yield was only about one-half that of the other sets. The pH values and analytical data on

the saps are given in table 4. The titration curves are shown in figure 6. The titration for the leaves from the high-Ca solution was not made.

In both stems and leaves, the  $\text{CaCO}_3$  treatment brought about an increase in pH in comparison with the high-Ca treatment. In the stems the low-Ca treatment increased the pH while in the leaves it caused a decrease. This may be explained by the fact that the sap from the stems had a higher concentration of K than was found in the sap from the

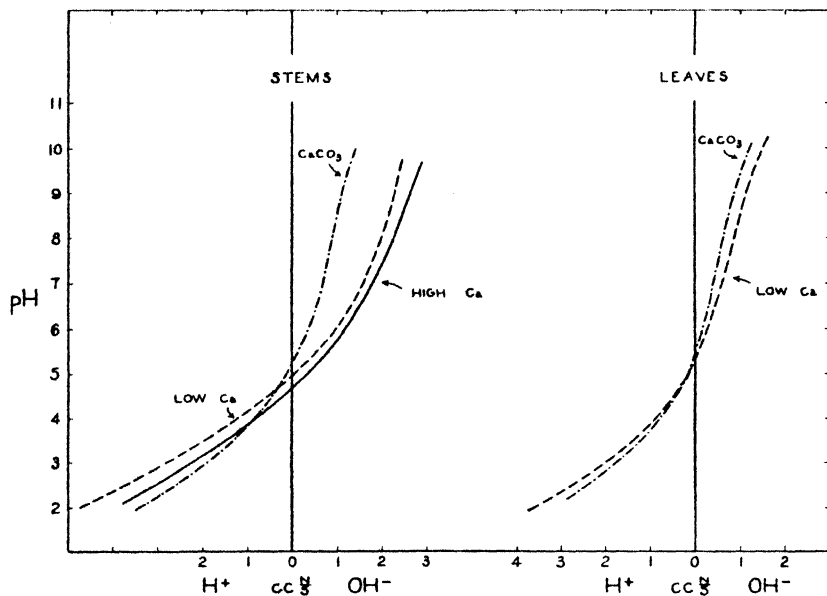


Fig. 6. Buffer curves of sap from buckwheat stems and leaves; plants grown with high Ca, low Ca, and  $\text{CaCO}_3$ . Five cc sap was used in titrations.

leaves, the substitution of K for Ca increasing the base content. The  $\text{CaCO}_3$  treatment resulted in a large decrease in buffer on both the acid and alkaline side, which would indicate that the organic-acid, including amino acid, content was decreased. The results of analyses made for K and oxalate in the sap are given in table 4. As usual, the K values of the low-Ca plants were higher than those of the high-Ca plants. There was a slight decrease of K in the sap of the  $\text{CaCO}_3$  plants. It was thought that in the  $\text{CaCO}_3$  series, more K may have entered the plant as  $\text{K}^+$  and  $\text{HCO}_3^-$  and that this would be reflected in the sap. Later evidence suggests that it is possible that even in this case more K was absorbed than by the high-Ca plants, but that a greater amount of it was precipitated.

The oxalate figures are more striking. The least amount of oxalate was present in the high-Ca set, twice as much being present in the low-Ca

set. This is probably owing to the extra equivalents of base absorbed as K in the latter set and available for combination with organic acids. In the  $\text{CaCO}_3$  set, there was evidently a marked reduction in the concentration of organic-acid radicals other than oxalic. Data cited later will show that large amounts of both Ca and oxalate were precipitated

TABLE 4

pH VALUES AND OXALATE AND POTASSIUM CONTENT OF SAP FROM BUCKWHEAT PLANTS GROWN IN SAND CULTURE\* UNDER DIFFERENT CONDITIONS OF CA SUPPLY

Culture conditions	pH value		$\text{C}_2\text{O}_4^{--}$		K		Yields, fresh weight for 10 jars		
	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Total
Low Ca .....	4.90	5.27	<i>p. p. m.</i> 2,360	.....†	<i>p. p. m.</i> 9,460	<i>p. p. m.</i> 5,930	<i>grams</i> 480	<i>grams</i> 120	<i>grams</i> 600
High Ca .....	4.66	5.40	940	.....†	7,540	.....†	430	100	530
$\text{CaCO}_3$ .....	5.20	5.62	3,280	.....†	7,310	5,970	230	55	285

Composition of solutions used

Solution	$\text{KNO}_3$	$\text{Ca}(\text{NO}_3)_2$	$\text{KH}_2\text{PO}_4$	$\text{MgSO}_4$	$\text{K}_2\text{SO}_4$	$\text{CaCO}_3$ per cent in sand	Fe, Mn, B
	<i>mols</i>	<i>mols</i>	<i>mols</i>	<i>mols</i>	<i>mols</i>	<i>per cent</i>	
Low Ca .....	0.0075	0.0005	0.001	0.001	0.001	0.0	To all solutions, B was added to give approximately 0.1 p.p.m. concentration and Mn 0.3 p.p.m. Fe as 0.5 per cent solution of tartrate was added as needed to maintain green color of plants.
High Ca .....	.0025	.0025	.001	.001	.002	0.0	
$\text{CaCO}_3$ .....	0.0075	0.0000	0.001	0.001	0.001	1.5	

\* Plants were grown in a greenhouse from April 29 to July 2, 1929; 2-gallon glazed earthenware crocks and pure silica sand were used.

† Analysis not made.

out of the sap, the greatest amount of both, in an insoluble form, being found in the plants from the  $\text{CaCO}_3$  set. It would seem that in the plants grown in the  $\text{CaCO}_3$  medium, oxalic acid was formed at the expense of other acids and that much of this oxalic acid was precipitated out, leaving a lower total acidity in the plant sap.

The results of the experiments on Ca may be briefly reviewed. If enough Ca is supplied for the maintenance of functions other than those concerned with the sap buffer system, injury to the plant may not result from a low-Ca supply, since sufficient base can be provided in the form of K. A low Ca supply usually causes an increase in organic-acid content of the sap as manifested by an increase in the buffer against acid. The change in buffer on the alkaline side is small but may show a slight decrease under conditions of low Ca supply. The actual pH of the sap

is not necessarily changed by this treatment. On the other hand,  $\text{CaCO}_3$  may produce considerable increase in pH. This change seems to be definitely unfavorable to the growth of buckwheat, and possibly of many other plants.  $\text{CaCO}_3$  also produces a large decrease in buffer on both sides of the neutral point in the case of buckwheat. Similar relations do not hold for melilotus.

It must be borne in mind that neither wheat nor buckwheat plants have, as a normal condition, large amounts of Ca in the sap. Buckwheat in its tissue as a whole often contains a large amount, but it is nearly all insoluble. For this reason no generalization can be made. Some plants which usually have a high Ca concentration in the sap show marked injury from a low-Ca treatment. This is the case with melilotus. The functions of Ca in plants of this type are not yet understood, but it does not appear from present evidence that the development of too great an acidity in the plant sap is the primary factor involved.

It was noted above that when the Ca supply was low there was an increased absorption of K by the plant, and a substitution in the sap of the former base by the latter. The reverse substitution may also occur, according to the results of experiments conducted in this laboratory. However, when the K supply is low, Ca, being a more slowly absorbed ion, is often not taken into the plant in sufficient quantities to permit complete substitution of bases. One result is that the pH of low-K plant sap is frequently slightly lower than that of high-K sap. The lower pH is generally accompanied by a large increase in the buffer against alkali. If the substances already suggested are responsible for the buffer in sap, this increased buffer indicates an increase in amides, amino acids, and sugars. Analytical data show this to be the case. Nightingale and coworkers<sup>(16)</sup> have also reported an increase in amide and amino nitrogen resulting from a low K supply, and many results of the same trend have been accumulated in this laboratory.

## THE OXALATE SYSTEM IN BUCKWHEAT

Crystals of calcium oxalate have been observed in many plants. The high content of Ca in buckwheat plants and the fact that most of it is in an insoluble form suggests the formation of this compound. As already mentioned, it is on this basis that the rôle of Ca in this and other plants with a similar type of metabolism has been thought to be that of precipitating the oxalic acid formed.

The data presented above have proved that a decrease in the Ca supply may not be accompanied by an increase in the hydrogen-ion concentration of the expressed sap. Furthermore, according to the data presented in table 4, the oxalate content of the sap from buckwheat stems was higher with the  $\text{CaCO}_3$  treatment than with the low-Ca treatment. It was therefore of interest to investigate more specifically the oxalate relations. In addition to calcium oxalate, some plant anatomists have reported crystals of potassium acid oxalate in plants. As the investigations on buffer systems had demonstrated that Ca could be more or less completely replaced by K, it was considered probable that oxalate might be converted into an insoluble form as potassium acid oxalate.

The solubility of Ca, K, and oxalate in water and in acid was investigated. Buckwheat plants were grown under controlled conditions of solution-culture technique. At harvest, the stems and leaves were separated and the plant tissues dried as quickly as possible at a temperature not exceeding  $90^\circ \text{C}$ . After being finely ground, different portions of the material were extracted with water, with 5 per cent HCl, and with hot 1 per cent HCl. It was observed that the constituents being investigated behaved in the same way under the last two treatments. The extraction was made with 25 parts of solvent to 1 of dry material, in an end-over-end shaker, for a period of 24 hours. The hot acid extract was made by heating for several hours on the steam bath.

The usual laboratory methods were employed for determinations of Ca and K. The following method for oxalate was developed: To the acidified aliquot to be analyzed 3 to 5 cc of 10 per cent  $\text{CaCl}_2$  were added, and the solution heated. This was followed by 10 cc of 20 per cent sodium acetate. The solution was then made just alkaline to methyl red by the addition of ammonia to the boiling solution. After a few minutes' boiling, 3 cc of acetic acid (1 part acetic acid, 4 parts water) were added. This was found to bring the solution to pH 5.0–5.2. The solution was filtered, preferably after standing, and washed well to remove soluble calcium. The precipitate containing calcium oxalate plus some organic matter was dissolved in HCl. The solution was then evaporated to dryness and the residue ignited. The Ca content of the ash was determined and from it the oxalate present in the sample calculated.

The results from analyses on buckwheat leaves grown under culture conditions of low Ca, high Ca, and  $\text{CaCO}_3$  are given in table 5. This set is similar to one discussed previously in connection with hydrogen-ion concentration and buffer titration. In columns 1 to 6 are given the amounts of each ion found in the extracts. In column 9 are given

the equivalents of water-insoluble oxalate minus water-insoluble Ca. On the assumption that the oxalate thus computed existed as  $\text{KHC}_2\text{O}_4$ , the equivalents of  $\text{HC}_2\text{O}_4^-$  would be only half the number expressed as  $\text{C}_2\text{O}_4^{--}$ . On this basis there is a suggestive agreement between the amounts of insoluble K and of residual oxalate.

TABLE 5

$\text{Ca}^{++}$ ,  $\text{K}^+$ , AND  $\text{C}_2\text{O}_4^{--}$  DISSOLVED FROM BUCKWHEAT STEMS BY DIFFERENT SOLVENTS

Culture conditions	Milliequivalents of certain ions for 100 grams dry weight										
	Soluble in H <sub>2</sub> O			Soluble in HCl*			Insoluble in water				
	C <sub>2</sub> O <sub>4</sub> <sup>--</sup>	Ca <sup>++</sup>	K <sup>+</sup>	C <sub>2</sub> O <sub>4</sub> <sup>--</sup>	Ca <sup>++</sup>	K <sup>+</sup>	C <sub>2</sub> O <sub>4</sub> <sup>--</sup>	Ca <sup>++</sup>	C <sub>2</sub> O <sub>4</sub> <sup>·-</sup> —Ca <sup>++</sup>	K <sup>+</sup>	HC <sub>2</sub> O <sub>4</sub> <sup>·-</sup>
	1	2	3	4	5	6	7	8	9	10	11
In leaves											
Low Ca .....	23	—†	35	128	75	46	105	75	30	11	15 0
High Ca .....	20	—†	30	120	86	38	100	86	14	8	7 0
CaCO <sub>3</sub> ....	31	—†	34	158	110	46	127	110	17	12	8 5
In stems											
Low Ca .....	20	1	114	62	34	125	42	33	.....	11	.....
High Ca .....	13	2	71	62	46	102	49	44	.....	31	.....
CaCO <sub>3</sub> .....	15	4	94	89	104	114	74	109	.....	20	.....

\* Cold 5 per cent acid was used for the stems and hot 1 per cent acid for the leaves.

† On the assumption that the oxalate computed by subtracting insoluble  $\text{Ca}^{++}$  from insoluble  $\text{C}_2\text{O}_4^{--}$  (col. 9) existed as  $\text{KHC}_2\text{O}_4$ , the  $\text{HC}_2\text{O}_4^-$  would be only half this oxalate.

‡ Amount negligible for present purpose.

The inference can reasonably be drawn that in buckwheat leaves oxalic acid formed in metabolism may be precipitated by either Ca or K. This is contrary to the contention that Ca in the form of  $\text{CaCO}_3$  is necessary for the precipitation of oxalic acid. It is also evident that not only is the total oxalate concentration highest in the  $\text{CaCO}_3$ -treated plants, but that the water-soluble oxalate is also highest in this set.

Analyses were also made on the stems of this set, the acid extract being made with cold 5 per cent  $\text{HCl}$ . The material was shaken for 24 hours, simultaneously with the water extract. The acid extract of the stems had a higher K and lower Ca content than that of the leaves and in this case also large amounts of both elements were insoluble in water (table 5). There are more than enough equivalents of Ca and K in an insoluble form to account for the oxalate precipitated. Insoluble compounds of these elements other than oxalates may be formed. The fig-

ures for the  $\text{CaCO}_3$  set show that this is undoubtedly the case for Ca, for even if the insoluble K is not included, there is still an excess of insoluble Ca over insoluble oxalate.

As before, the total oxalate was highest in the plants receiving the  $\text{CaCO}_3$  treatment. It would seem that the excess oxalate was formed in response to the presence of  $\text{CaCO}_3$  in the medium, rather than that the Ca was essential to precipitate the oxalic acid necessarily formed as a result of metabolic processes.

It is of interest that in this experiment slightly more growth was made by the low-Ca plants than by the high-Ca plants. The plants grown in the medium containing  $\text{CaCO}_3$  had only about half the weight of those grown in the other media (table 4). In both stems and leaves the concentration of water-soluble oxalate was higher in the low-Ca set than in the high, and in the stems it is highest of all in the low-Ca plants. This seems to refute the idea that oxalate is injurious to growth of this type of plant. In both stems and leaves, water-soluble K is highest in the low-Ca plants. It is probable, therefore, that as long as sufficient base is present, an increase in oxalate is not injurious.

It might be argued that the extra amount of K found in the water extract of the plant tissues was responsible for the increased growth, regardless of the oxalate concentration. It will be observed, however, that both water-soluble and total K are higher in the  $\text{CaCO}_3$  plants than in the high-Ca plants, and yet no increase in growth resulted. This is not conclusive, for the  $\text{CaCO}_3$  may have counteracted the beneficial effect of the K absorbed. As suggested above, it is possible that the additional K was absorbed in this case as  $\text{K}^+$  and  $\text{HCO}_3^-$ , and that this was in part responsible for the alkalinity observed in the expressed sap of the  $\text{CaCO}_3$  plants.

Another experiment was conducted with culture solutions, using three solutions: (1) high Ca and K, (2) low Ca, and (3) low K. The dried material was extracted with water and 5 per cent HCl. The analyses are given in table 6. In the low-K set all the potassium is in a water-soluble form. There is more insoluble Ca than insoluble oxalate, indicating that some Ca exists in other insoluble forms. In the low-Ca plants, there is a comparatively small amount of insoluble oxalate present. In this case the equivalents of insoluble K alone far exceed those of insoluble oxalate, suggesting that some K may go out of solution in some form other than  $\text{KHC}_2\text{O}_4$ . It may be that precipitation as  $\text{KH}_3(\text{C}_2\text{O}_4)_2$  occurs in some cases, which would make the discrepancy still greater. Again in this set, the highest total oxalate concentration was associated with the highest Ca content.

In the low-Ca plants, at the pH of the sap, the soluble oxalate would be present, if in equilibrium with K, as  $K_2C_2O_4$ . Assuming this equilibrium to exist, there would not be enough water-soluble K to form salts with the oxalate ions present. As the Mg was probably very low, being supplied in small amount, it is possible that there was an accumulation of oxalic acid with lowering of pH. The fact that in this experiment the yield from the low-Ca set was less than the high-Ca set, strengthens this assumption.

TABLE 6

$Ca^{++}$ ,  $K^+$ , AND  $C_2O_4^{--}$  DISSOLVED FROM BUCKWHEAT STEMS BY DIFFERENT SOLVENTS

Culture conditions*	Milliequivalents of certain ions for 100 grams dry weight									Yields, dry weight, for 36 jars		
	Soluble in H <sub>2</sub> O			Soluble in HCl†			Insoluble in H <sub>2</sub> O					
	C <sub>2</sub> O <sub>4</sub> <sup>--</sup>	Ca <sup>++</sup>	K <sup>+</sup>	C <sub>2</sub> O <sub>4</sub> <sup>--</sup>	Ca <sup>++</sup>	K <sup>+</sup>	C <sub>2</sub> O <sub>4</sub> <sup>--</sup>	Ca <sup>++</sup>	K <sup>+</sup>	Stems	Leaves	Total
Low Ca	62	—†	41	81	21	94	19	21	53	grams 525	grams 185	grams 710
High K	4	5	34	111	104	73	107	99	39	695	250	945
Low K	5	46	9	159	232	9	154	186	0	480	240	720

Composition of culture solutions used

Solution	$KNO_3$	$Ca(NO_3)_2$	$KH_2PO_4$	$MgSO_4$	$K_2SO_4$	Fe, Mn, B
	mols	mols	mols	mols	mols	
Low Ca	0 005	0 0002	0 0004	0 0004	0 0006	Treatment similar to that described in table 4
High K	005	0050	0004	001	0000	
Low K	0 000	0 0050	0 0004	0 001	0 0000	

\* Plants were grown in a greenhouse from June 28 to July 22, 1929 2-liter jars were used with 2 plants in each jar

† Cold, 5 per cent acid

[ ] ‡ Amount negligible

The theory that absorption of Ca is necessary in order to precipitate oxalic acid formed in plant metabolism, is not substantiated. It would appear to be immaterial whether the oxalate is in a soluble or insoluble form, provided sufficient base, either Ca or K, is present. Furthermore, it is shown that when the Ca content of buckwheat plants is markedly increased by growing them either in the presence of an excess of  $CaCO_3$ , or in a low-K solution, there is a decided increase in the amount of oxalate formed. An increased yield of oxalate may possibly result from an upsetting of metabolism caused by these treatments. On the other hand, plants grown in a solution low in Ca do not necessarily show a similar increase of oxalate.



## SUMMARY

In the light of previous investigations conducted by the author, a further attempt was made to identify the principal types of substances responsible for the buffer of plant saps. All work was done on sap expressed from tissues frozen and thawed. It is concluded that organic acids, amides, amino acids, phosphates, and sugars are the substances of most importance. The data suggest that in studies on plant metabolism, titration curves may be a useful means of ascertaining large changes in some of the organic constituents of the sap.

A low phosphate supply resulted in an increase of hydrogen-ion concentration and buffer in the sap. This probably indicates an increase in organic acids, amides, amino acids, and possibly sugars. Similar changes may occur as a result of low K supply.

The importance of Ca in the plant buffer system was studied. It is shown that Ca is not an indispensable part of the buffer system of the plants studied. Such plants when grown with a low Ca supply do not necessarily show an increase of hydrogen-ion concentration in the sap, nor are they always injured. Under these conditions, the base necessary for the buffer system is supplied by an increased absorption of K. On the other hand,  $\text{CaCO}_3$  treatment may produce a condition of alkalinity which is definitely injurious to some plants. Furthermore,  $\text{CaCO}_3$  was not found to cause a decrease in the oxalate content of plant sap in buckwheat.

The oxalate system in buckwheat was investigated. A large proportion of the oxalate is usually in an insoluble form. The data indicate that this may be precipitated either with Ca or K. The theory that  $\text{CaCO}_3$  or  $\text{Ca}(\text{HCO}_3)_2$  is necessary for the neutralization of organic acids in such plants is not substantiated. The high Ca content undoubtedly indispensable for good growth of certain types of plants seems to require some other explanation.

## ACKNOWLEDGMENT

The author wishes to acknowledge suggestions received from Professor D. R. Hoagland during the progress of the experiments and the writing of the manuscript.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

NOVEMBER, 1932

No. 6

## VACCINATION OF SWINE AGAINST TUBERCULOSIS WITH CALMETTE-GUÉRIN CULTURE, BCG<sup>1</sup>

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### INTRODUCTION

Swine being highly susceptible to bovine tuberculosis and, under many systems of swine husbandry, exposed to infectious material from tuberculosis cattle, it was thought advisable to carry out certain experiments at the University of California to test the protective effect of the Calmette-Guérin culture known as BCG, upon hogs under controlled exposures to infection. Results of similar experiments conducted with cattle have already been published by the writers.<sup>(5)</sup>

### REVIEW OF LITERATURE

At the time the experiments reported herein were started no publications were known to the writers regarding the immunizing effect of BCG on swine, and contributions appearing since that time are not in agreement.

Ascoli<sup>(1, 2)</sup> and his collaborators reported an experiment with 12 pigs, 6 of which were vaccinated before the tenth day of age. The other 6 were retained unvaccinated as controls. Of the 6 vaccinated pigs, 3 were given the vaccine by mouth, each receiving a dose of 10 mg on three alternate days. The other 3 pigs were vaccinated by injecting each sub-

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cutaneously behind the ear with one dose of 10 mg. In 1 of these latter (No. 13) the effect was considered unsatisfactory by Ascoli because it showed sloughing at the point of injection. The test infection was given twelve weeks later by injecting into the ear vein a suspension of virulent bovine tubercle bacilli. Autopsy notes were published only for 3 of the vaccinated pigs and 1 of the controls. These 4 cases, in which each received 1 mg of virulent bacilli intravenously August 5, 1926, may be summarized as follows:

Pig No.	Vaccinated	Date slaughtered	Tuberculous lesions
19	Subcutaneously 10 mg on May 15, 1926	March 10, 1927	Slight in bronchial and mediastinal lymph nodes
13	Subcutaneously 10 mg on May 11, 1926	June 30, 1927	Tubercles marked in bronchial, mesenteric, hepatic and pharyngeal lymph nodes and in liver; slight in kidney
2	By mouth 10 mg doses on May 11, 13, and 15, 1926	June 30, 1927	Moderate lung lesions, extensive lymphatic lesions; more extensive liver lesions than No. 13
4	Control	June 22, 1927	Extensive in lungs and lymphatics

Four of the other pigs had received 0.02 mg virulent tubercle bacilli intravenously and 4 only 0.0005 mg. Ascoli did not state what was found when these 8 swine were butchered except that No. 20 which had been vaccinated subcutaneously and then infected intravenously with 0.0005 mg came nearest to the degree of resistance exhibited in No. 19. He concluded that vaccination by mouth had failed to develop any perceptible resistance to the particular kind of test infection which he had used. On the other hand he stated that the results in the swine vaccinated subcutaneously justified the conclusion that this method is capable of "pre-munizing" swine against tuberculous infection.

From these two publications by Ascoli, the writers can not see how he and his coworkers are justified in such a conclusion, particularly since this was apparently based on the relatively slight lesions found in his swine No. 19. It should be noted that this swine was slaughtered 104 days before the control and it seems to the writers that if it had been permitted to live until June 30, 1927, the lesions might have developed to the extent of those found in control No. 4 or the pig (No. 2) which had been vaccinated by mouth.

Sanz<sup>(8)</sup> in Chile has reported the vaccination of 993 pigs, but stated he had never seen any signs of tuberculosis following vaccination. Most

of his experiments were apparently field trials without the use of unvaccinated controls. However, he states that in herds of swine badly infected with tuberculosis through the ingestion of milk from tuberculous cows, the systematic vaccination of all newborn pigs caused an arrest of the tuberculous infection while the older swine not vaccinated continued to die of tuberculosis.

Jundell and Magnusson<sup>(7)</sup> carried out an experiment with 24 pigs. Eight of them were vaccinated when 14 to 16 days old by injecting 10 mg BCG subcutaneously near the point of the breast bone; eight others when 3 to 9 days old were given 10 mg doses by mouth on three alternate days; and eight were retained as controls.

After two months the 16 vaccinated pigs and 6 of the controls were subjected to infection by feeding to each animal sweet milk mixed with 50 cc of a thick puree obtained by grinding udder lesions of a tuberculous cow. It was estimated that each pig received about 2 billion tubercle bacilli. The 2 controls which were not fed this material were retained on the same premises.

In 19 to 22 weeks the entire 24 swine were slaughtered and all found to be tuberculous except the 2 controls which had not eaten the tuberculous udder tissue. Meat inspectors condemned as unfit for food 3 of the controls, 4 of those vaccinated by mouth, and 4 of those vaccinated subcutaneously. The investigators stated that scarcely a perceptible difference existed in the extent of tuberculosis between the control and the vaccinated animals and no indications of protection against tuberculosis in swine were attributable to the vaccine. However, their experiments on calves with BCG vaccine prepared in a similar way to that used on the pigs had exercised a preventive action against tuberculosis in 50 per cent of the calves. The pigs vaccinated subcutaneously developed an induration at the point of injection which was distinct at the end of the first month, but had completely disappeared by the end of the second month. At the time of slaughter no lesions were visible at the inoculation points. From this they concluded since none of the 8 pigs vaccinated subcutaneously had developed an abscess, that swine in general do not have so acute a local reaction toward BCG as do larger animals.

Also according to Jundell and Magnusson an experiment by Jerlov<sup>(8)</sup> was made August 31, 1926, by injecting a 14-day-old pig subcutaneously with 50 mg BCG; a pig of similar age served as a control. Six days after vaccination, tumefaction at the point of inoculation had disappeared, and no reaction was observed afterwards at this point. On September 23 the tuberculin test by the intracutaneous method gave negative results in both animals, but on October 9 the vaccinated animal gave a manifest

reaction to tuberculin, while the control was still negative. Beginning October 17 both animals were fed at six different times with fragments of lungs from a tuberculous cow. After a time they began to lose weight, especially the vaccinated pig. They were slaughtered at the age of six months, and both presented very advanced and generalized tuberculosis. In the vaccinated animal the lesions had especially a progressive character and the lymphatic glands were fused, while in the control they were in part calcified. Jerlov considered the infective doses were too massive, and did not therefore wish to draw conclusions.

In laboratory experiments at Utrecht, de Blicke<sup>(4)</sup> found that the resistance of the calves was increased by BCG vaccination to a greater extent than that of pigs, but he did not think the vaccination gave a specific immunity.

### EXPERIMENTAL METHODS USED

In the main, the experimental procedures with swine reported herewith followed closely those carried out on cattle at the California station,<sup>(5)</sup> especially in relation to the technique of preparing and administering the vaccine, forms of exposure to infection, and autopsy methods.

*Source of Pigs Used in the Experiments.*—The animals used in the experiments were all secured from sources believed to be free from tuberculosis, and in addition were tuberculin-tested by intradermic injection into an ear of 5 per cent solution of precipitated tuberculin in sterile distilled water. In most cases the swine were farrowed by tuberculosis-free sows on noninfected premises of the University campus at Davis and kept free from any contact with tuberculous animals from birth until the time of artificial infection. All were vaccinated against hog cholera with serum and virus at approximately six weeks of age or at a time to allow them to be fully over the effects before exposure to tuberculosis. The pigs vaccinated with BCG were kept separated from those to be used as controls until exposure, when all were allowed to run together.

*Preparation of the Vaccine.*—The original cultures of BCG used in the experiments were obtained by one of the writers (Traum) at the Pasteur Institute, Paris, on April 7, 1926. Care has been taken to grow and prepare the vaccine and test it for pathogenicity on guinea pigs in exactly the way prescribed in directions received from Calmette.<sup>(3)</sup> The stock cultures have been maintained in Roux tubes on potato with 5 per cent glycerine broth for a series of nine generations. The tenth

and eleventh generations have been propagated on 5 per cent glycerinated ox-bile potato and then replanted on potato in glycerine broth for nine generations. Old, fully-ripened potatoes are used, because it has been observed that media made from new potatoes give only a feeble growth of BCG. Sauton's medium has been used for propagating some of the vaccine serials. A separate incubator planting room and equipment have been used exclusively for BCG culture and vaccine preparation. The vaccine has been made from cultures not less than 19 days nor more than 26 days old. When ready for use, each cubic centimeter of vaccine contained 10 mg of BCG bacilli (weight after removal of excess moisture by blotting with filter paper), suspended in a sterile diluent consisting of 100 parts of distilled water, 1 part of chemically pure glucose, and 1 part of chemically pure glycerine, as prescribed by Calmette.<sup>(3)</sup>

*Experimental Groups.*—The objects of the investigations were to test the value of BCG as an immunizing agent against tuberculosis in swine, the length of time after vaccination that protection, if any, developed, and to determine the best methods and ages for vaccination. There are, therefore, included in the records to be presented the following groups: (1) Fourteen pigs that were subcutaneously vaccinated with 100 mg of BCG at ages varying from 3 days to 5 days and exposed to feeding infection at 27 or 93 days after vaccination, and with an equal number of controls, were killed for examination at intervals varying from 90 to 297 days after the first exposure (tables 2A and 2B). (2) Eight that were subcutaneously vaccinated with 100 mg of BCG at ages varying from 65 days to 171 days and exposed to feeding infection at 18 or 140 days after vaccination, and with 4 controls, were killed for examination from 129 to 176 days after the first exposure (tables 3A and 3B). (3) Five that were intramuscularly vaccinated with 100 mg of BCG, exposed 28 or 34 days thereafter to infection with milk from a cow with tuberculosis of the udder, and with 6 controls, were killed at 36 and 74 days after the first milk feeding (table 4). (4) Six that were subcutaneously vaccinated with 100 mg of BCG at 105 days of age, exposed to infection by intravenous injection of a virulent culture of bovine tuberculosis 60 days thereafter, and with 5 controls, were killed from 237 to 243 days after injection of the culture (table 5). (5) Two that were vaccinated intravenously with 1.0 mg of BCG at 93 days of age, exposed to feeding infection 62 days thereafter, and with 3 controls, were killed 165 days after the first exposure (table 6). (6) Four intradermally vaccinated at the ages of 7, 8, and 93 days with 50 mg of BCG, exposed to feeding infection 60 or 62 days thereafter, and with an equal number of



controls, were killed from 165 to 200 days after first exposure (tables 7 and 8). (7) Six which were given three doses of BCG by mouth at the age of 7 and 8 days, exposed to feeding infection 60 days thereafter, and with 5 controls, were killed between 185 and 200 days after first exposure (table 9).

*Autopsy Methods and Guinea Pig Injections.*—All of the swine were slaughtered in small local slaughter establishments where official inspection was maintained, with the exception of those that showed physical indications of tuberculosis. These were killed in the University laboratories at Davis. In either case all of the organs and the principal body glands were carefully examined for lesions by slicing thin sections. When lesions were present some of those from the head, the thoracic and abdominal cavities, and occasionally from other areas, were removed without slicing for guinea pig inoculation. If no lesions could be seen the apparently normal tissues in these parts were used for guinea pig injections. The usual procedure was to inject guinea pigs with material from head, bronchial, gastrohepatic, and mesenteric glands, and from lungs, spleen, and liver, whether lesions were present or not. Before injection of any tissues removed from the organs, or from any lymph nodes, the tissues were first immersed in boiling water from 10 to 12 seconds and carefully and thinly sectioned with sterilized scissors for observation of any small lesions in the apparently healthy tissues. These sections were ground in sterile mortars with physiological sodium chloride solution, examined by smears for acid-fast bacteria and from 1.0 to 2.0 cc injected intramuscularly into guinea pigs. The guinea pigs were usually killed between 60 and 90 days after inoculation.

## SUBCUTANEOUS VACCINATION AND INFECTION EXPOSURE BY FEEDING

*Method of Vaccination.*—All of the swine in this group received 100 mg of BCG vaccine suspended in 10 cc of diluent, the preparation of which has been previously described. The injections were made with an 18-gauge hypodermic needle into the subcutaneous fascia of the flank. In all animals, except pig No. 9 (table 2A), 5 cc were injected in each flank; No. 9 had the entire 10 cc (100 mg) introduced into the left flank.

*Character of Infectious Material and Method of Feeding.*—The tissues fed were obtained from tuberculous cattle condemned at an abattoir or from guinea pigs, rabbits, and hogs infected with bovine tuberculosis. The character of the material, approximate quantity fed, and microscopic and guinea pig tests are outlined in table 1.

TABLE 1  
SOURCES AND AMOUNTS OF TUBERCULOUS TISSUE USED TO INFECT SWINE BY  
FEEDING\*

Pig Nos. and number of feedings	Date collected	Description of material	Dates fed	Dose for each pig		Guinea pig control result†
				Grams of tissue‡	Estimated bacilli‡	
1-14 and 101-114 (10 feed- ings)	Aug. 12 (1927)	Tuberculous tissue from the lungs, costal pleura, and lymph nodes of 2 aged cows and a calf, 7 months old.....	Aug. 13	2.0	500,000	+
			Aug. 15	2.0	500,000	+
			Aug. 17	2.0	500,000	+
	Aug. 18	Composite sample of tuberculous tissues from an aged cow.....	Aug. 19	2.0	Positive smears	+
			Aug. 22	2.0	Positive smears	+
			Aug. 23	2.0	Positive smears	+
	Aug. 26	Tuberculous tissue from 2 rabbits and 2 guinea pigs.....	Aug. 28	1.0	1,320,000	+
			Sept. 2	1.0	1,650,000	+
	Sept. 7	Lung of calf No. 101 which died of miliary tuberculosis following intravenous injection of virulent culture....	Sept. 9	0.5	Positive smears	+
			Sept. 10	0.5	Positive smears	+
17-22 and 116-118 (30 feed- ings)	Aug. 12 (1927)	Tuberculous tissue from the lungs, costal pleura and lymph nodes of 2 aged cows and a calf, 7 months old.....	Aug. 13	10.0	500,000	+
			Aug. 15	10.0	500,000	+
			Aug. 17	2.0	690,000	+
	Aug. 16	Tuberculous tissue from the lungs, costal pleura and lymph nodes of an aged cow.....	Aug. 19	2.0	690,000	+
			Aug. 22	2.0	690,000	+
			Aug. 23	2.0	690,000	+
	Aug. 26	Tuberculous tissue from 2 rabbits and 2 guinea pigs.....	Aug. 27	2.0	1,320,000	+
	Sept. 1	Tissues from lungs and lymph nodes of a range steer.....	Sept. 2	2.0	850,000	+
	Sept. 7	Lung of calf No. 101 which died of miliary tuberculosis.....	Sept. 8	0.5	1,890,000	+
			Sept. 9	0.5	1,890,000	+
	Sept. 10	Lymph node tissue, calf No. 101.....	Sept. 11	2.0	960,000	+
			Sept. 12	2.0	960,000	+

\* Other forms of infection used are described under their respective headings in the text.

† This represents the weight of tuberculous tissue after grinding but before straining through several thicknesses of cheese cloth. The actual amount of original tissue in each dose is less than one-third of this weight.

‡ Made by suspending 10 or 20 grams of thoroughly ground tuberculous tissue in a measured quantity of physiological sodium chloride solution; then estimating according to the technique for the direct microscopic counting of bacteria in milk. The estimate was only comparative. The number of organisms consumed was probably far greater than indicated in this column, since the character of the tissue and the method of estimating did not lend themselves to more definite determination of the number of organisms.

§ The inoculum for the guinea pigs was a portion of the ground and strained tuberculous materials suspended in milk as fed to the calves and the weight is given in terms of ground but unstrained material.

TABLE 1—(Continued)

Pig Nos. and number of feedings	Date collected	Description of material	Dates fed	Dose for each pig		Guinea pig control result†
				Grams of tissue‡	Estimated bacilli‡	
17-22 and 116-118 (30 feed- ings) (Cont'd)	Sept. 13 (1927)	Lung of calf No. 102, which died of mil- itary tuberculosis .....	Sept. 14 Sept. 16	5.0 5.0	1,950,000 1,950,000	+ +
	Sept. 13	Lung and lymph nodes, calf No. 102...	Sept. 18 Sept. 19	0.3 0.3	2,000,000 2,000,000	+ +
	Sept. 23	Tuberculous viscera from guinea pigs that had been inoculated with the feeding material used on Aug. 13, as shown in this table .....	Sept. 24 Sept. 28	1.0 0.5	2,040,000 1,020,000	+ +
	Sept. 28	Caseous material from tuberculous le- sions in the lung and lymph nodes of an aged cow .....	Sept. 29 Sept. 30	0.5 0.5	5,850,000 5,850,000	+ +
			Oct. 1	0.5	5,850,000	+
			Oct. 2	0.5	5,850,000	+
			Oct. 3	0.5	5,850,000	+
			Oct. 4	0.5	5,850,000	+
			Oct. 5	1.11	12,980,000	+
			Oct. 6	1.11	12,980,000	+
			Oct. 7	1.11	12,980,000	+
			Oct. 8	1.11	12,980,000	+
	Oct. 10	Tuberculous viscera of 14 guinea pigs inoculated from feedings Sept. 2-8 shown in this table .....	Oct. 11 Oct. 12	1.11 1.11	12,980,000 12,980,000	+ +
15-16 and 115 (20 feed- ings)	Sept. 23 (1927)	Tuberculous viscera from guinea pigs that had been inoculated with the feeding material used on Aug. 13, as shown in this table .....	Sept. 24 Sept. 28	1.0 0.5	2,040,000 1,020,000	+ +
	Sept. 28	Caseous material from tuberculous le- sions in the lung and lymph nodes of an aged cow .....	Sept. 29 Sept. 30	0.5 0.5	5,850,000 5,850,000	+ +
			Oct. 1	0.5	5,850,000	+
			Oct. 2	0.5	5,850,000	+
			Oct. 3	0.5	5,850,000	+
			Oct. 4	0.5	5,850,000	+
			Oct. 5	1.11	12,980,000	+
			Oct. 6	1.11	12,980,000	+
			Oct. 7	1.11	12,980,000	+
			Oct. 8	1.11	12,980,000	+
	Oct. 10	Tuberculous viscera of 14 guinea pigs inoculated from feedings, Sept. 2-8 shown in this table .....	Oct. 11 Oct. 12	1.11 1.11	12,980,000 12,980,000	+ +
	Oct. 13	Tuberculous viscera of 8 guinea pigs inoculated with bovine lesions .....	Oct. 14 Oct. 15	1.8 1.8	45,000,000 45,000,000	+ +
	Oct. 15	Tuberculous viscera of 16 guinea pigs inoculated with feedings, Sept. 9-14, shown in this table .....	Oct. 16 Oct. 17	4.8 4.8	67,500,000 67,500,000	+ +
	Oct. 18	Tuberculous viscera of 21 guinea pigs inoculated with feedings Sept. 14-19, shown in this table .....	Oct. 19	6.8	73,100,000	+
	Oct. 19	Viscera of 3 guinea pigs and a rabbit which had died of tuberculosis after injection with bovine lesions .....	Oct. 20	11.0	3,500,000,000	+

TABLE 1—(Concluded)

Fig Nos. and number of feedings	Date collected	Description of material	Dates fed	Dose for each pig		Guinea pig control result¶
				Grams of tissue†	Estimated bacilli‡	
34-35, 38-39, and 134-139 (5 feed- ings)	Aug. 24 (1928)	Tuberculous lymph nodes from a cow and a pig obtained from slaughter house.....	Aug. 25	13.2	One acid-fast per 80 fields	+
	Aug. 31	Tuberculous lymph nodes from several dairy cows, killed at local slaughter house.....	Aug. 31	16.0	160,000	+
			Sept. 1	16.0	160,000	+
	Sept. 4	Lungs from a dairy cow killed at a local abattoir, extensive tuberculosis with much caseous material.....	Sept. 5	43.0	154,000,000	+
			Sept. 6	43.0	154,000,000	+
36-37, 40-45, and 140-144 (4 feed- ings)	May 31 (1928)	Tuberculous lesions from control pigs 112 and 113, killed on May 31, 1928.....	June 1	17.0	Positive smears	No data
			June 2	17.0	Positive smears	No data
	June 6	Lesions from 5 tuberculous guinea pigs that had been injected with lesions from vaccinated pigs 10 and 11, and lesions from control pig 114.....	June 6	7.4	Positive smears	+
	June 20	Lesions from 4 tuberculous guinea pigs that had been injected with lesions from controls 110 and 111.....	June 20	4.8	Positive smears	+

\* Other forms of infection used are described under their respective headings in the text.

† This represents the weight of tuberculous tissue after grinding but before straining through several thicknesses of cheese cloth. The actual amount of original tissue in each dose is less than one-third of this weight.

‡ Made by suspending 10 or 20 grams of thoroughly ground tuberculous tissue in a measured quantity of physiological sodium chloride solution; then estimating according to the technique for the direct microscopic counting of bacteria in milk. The estimate was only comparative. The number of organisms consumed was probably far greater than indicated in this column, since the character of the tissue and the method of estimating did not lend themselves to more definite determination of the number of organisms.

§ The inoculum for the guinea pigs was a portion of the ground and strained tuberculous materials suspended in milk as fed to the calves and the weight is given in terms of ground but unstrained material.

The tuberculous tissues were ground in a meat-grinding machine, weighed after grinding, mixed thoroughly with sufficient warm milk to allow for 100 cc to be fed to each pig, and strained through a single layer of cheese cloth. In practically every case each pig was individually allowed to drink from a pan 100 cc of such mixtures at each feeding. The number of tubercle bacilli in the mixtures naturally varied from time to time. The periods of feeding also varied from daily to intervals of several days. The period of those receiving 10 feedings extended from August 13, 1927, to September 10, 1927; those receiving 20 feedings from September 24, 1927, to October 20, 1927; and those receiving 30 feedings from August 13, 1927, to October 12, 1927.

**TABLE 2A**  
**RESULTS OF SUBCUTANEOUS VACCINATION OF SUCKLING PIGS WITH 100 MG BCG AND INFECTION EXPOSURE BY TEN FEEDINGS OF TUBERCULOUS TISSUES**  
 (For controls see table 2B)

Pig No.	Intervals in days			Size of vaccination nodule in mm	Tuberculous lesions*								Guinea pig inoculations	Remarks
	Birth vaccination	Vaccination to first exposure	First exposure to autopsy		Cervical	Tracheal	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver	Acid-fast bacilli in smears	
1	3	27	90	7 x 7	X	-	X	-	-	-	-	/	-	D† Killed because moribund, pneumonia. Liver contained several white areas on surface, 1 mm diameter. Necrotic enteritis.
2	3	27	138	15 x 15	X	X	/	/	/	-	X	/	-	Liver studded with tubercles.
3	3	27	138	10 x 15	X	X	/	/	/	-	X	/	-	Parotid node enlarged 4X, and caseous.
4	3	27	162	3 x 5	X	X	/	/	/	-	X	/	-	.....
5	3	27	210	2 x 2	X	X	/	/	/	-	X	/	-	.....
6	5	93	138	5 x 8	X	X	/	/	/	-	X	/	-	.....
7	5	93	138	?	-	X	/	/	/	-	X	/	-	Left parotid contained caseous foci.
8	5	93	202	.....	-	/	X	/	X	-	-	/	-	Lymph nodes that were apparently normal also produced tuberculosis in guinea pigs.
9	5	93	238	.....	-	/	X	/	X	-	-	/	-	About 7 months after exposure, became lame in hind leg, followed by posterior paralysis. At autopsy the twelfth thoracic and third lumbar vertebrae were found badly necrosed with tuberculosis.
10	5	93	259	2 x 2	-	/	/	/	X	-	-	/	-	Lesions from submaxillary and ileocecal lymph nodes produced tuberculosis in guinea pigs.
11	5	93	259	2 x 2	-	/	/	/	X	-	-	/	-	.....
12	5	93	292	3 x 2	/	/	X	/	X	-	-	/	-	Left prescapular enlarged 3X, and contained caseocalcarous foci.
13	5	93	297	?	/	/	X	/	X	-	-	/	-	.....
14	5	93	297	1 x 1	/	/	/	/	-	-	-	-	-	.....

\* The symbols for lesions are adopted from charts used by federal meat inspectors and the names of the various groups and individual lymph nodes or lymph glands correspond in the main to those given by Buckley and Casar. The adjectives bronchial, mesenteric, etc., referring to lymph nodes are, for brevity, used without the words "lymph nodes." Definitions for *slight*, *well marked*, *extensive*, and *no lesions* have been formulated by the writers as a general guide as follows:

*Slight*—Caseous or caseocalcarous isolated areas less than 15 mm in diameter.  
*Well marked*—A single caseous or caseocalcarous area, 15 to 30 mm in diameter.  
*Extensive*—All progressive or multiple milium tubercles, any one lesion over 30 mm in diameter, or two or more lesions over 25 mm each.

— *Negative*—No lesions found.

† Does not include smears from vaccination lesion.

‡ "D" indicates that guinea pigs died prematurely.

TABLE 2B  
RESULTS IN NONVACCINATED PIGS EXPOSED TO INFECTION BY TEN FEEDINGS OF TUBERCULOUS TISSUES

Pig No.	Interval in days		Tuberculous lesions*							Acid-fast bacilli in smears	Guinea pig inoculations†	Remarks
	Birth to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-ocolic	Gastrohepatic	Lungs	Spleen	Liver		
101	30	49	-	-	-	-	-	-	-	-	0	Died; necrotic enteritis present. No vaccinated hog killed at corresponding time.
102	30	112	-	-	-	-	-	-	-	-	0	Died; record of autopsy shows only extensive generalized tuberculosis.
103	30	128	-	-	-	-	-	-	-	-	0	Omentum a mass of tuberculous foci. Died in emaciated condition.
104	30	162	-	-	-	-	-	-	-	-	+	Left prescapular lymph nodes enlarged 2X, and caseous.
105	30	210	-	-	-	-	-	-	-	-	+	Right and left inguinals enlarged 2X, and caseous. Costal pleura had 18 to 20 typical nodules 1 to 3 mm in diameter.
106	98	138	-	-	-	-	-	-	-	-	+	Prescapular lymph nodes contained caseous nodules 3 mm in diameter.
107	98	138	-	-	-	-	-	-	-	-	+	.....
108	98	202	-	-	-	-	-	-	-	-	D	All five guinea pigs injected died within 72 hours.
109	98	238	-	-	-	-	-	-	-	-	+	.....
110	98	257	-	-	-	-	-	-	-	-	+	Many tubercles on parietal pleura, 0.5 to 2.0 cm in diameter.
111	98	257	-	-	-	-	-	-	-	-	+	Lame in right hind leg a few days before killed. Right stifle joint and last lumbar vertebra tuberculous. Precrural and inguinal lymph nodes showed foci.
112	98	292	-	-	-	-	-	-	-	-	+	.....
113	98	292	-	-	-	-	-	-	-	-	+	.....
114	98	297	-	-	-	-	-	-	-	-	+	.....

\* For explanation of symbols see footnote of table 2A.

† "D" indicates that guinea pigs died prematurely. "0" indicates that no guinea pigs were injected.

TABLE 3A  
RESULTS OF SUBCUTANEOUS VACCINATION OF PIGS WITH 100 MG BCG AND INFECTION EXPOSURE BY TWENTY TO THIRTY FEEDINGS  
OF TUBERCULOUS TISSUES  
(For controls see table 3B)

Pig No.	Number of feedings	Interval in days			Size of vaccination nodule in mm	Tuberculous lesions*								Acid-fast bacilli in smears†	Guinea pig inoculations	Remarks
		Birth to vaccination	Vaccination to first exposure	First exposure to autopsy		Cervical	Tracheal	Mesenteric	Cecal-colic	Gastro-hepatic	Lungs	Spleen	Liver			
15	20	171	18	129	?	×	×	×	×	×	×	×	×	+	+	Carpus of right leg enlarged 2X, and tuberculous. Prepectoral lymph nodes caseous, 50 x 40 x 30 mm.
16	20	171	18	132	?	×	—	—	—	—	—	—	—	+	+	.....
17	30	148	77	173	?	×	—	—	—	—	—	—	—	+	+	.....
18	30	148	77	173	?	×	—	—	—	—	—	—	—	+	+	.....
19	30	65	98	173	—	×	—	—	—	—	—	—	—	+	+	.....
20	30	85	98	176	15 x 20	—	—	—	—	—	—	—	—	+	+	.....
21	30	169	129	134	—	—	—	—	—	—	—	—	—	+	+	.....
22	30	158	140	175	?	—	—	—	—	—	—	—	—	+	+	.....

\* For explanation of symbols see footnote of table 2A.  
† Does not include smears from vaccination lesions.

TABLE 3B  
RESULTS IN NONVACCINATED PIGS EXPOSED TO INFECTION BY TWENTY TO THIRTY FEEDINGS OF TUBERCULOUS TISSUES

Pig No.	Number of feedings	Interval in days		Tuberculous lesions*								Acid-fast bacilli in smears	Guinea pig inoculation	Remarks
		Birth to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver	Other lesions		
115	20	189	132	×	×	×	×	×	×	—	—	—	—	.....
116	30	225	173	×	—	—	—	—	—	—	—	—	—	.....
117	30	225	173	×	—	—	—	—	—	—	—	—	—	.....
118	30	298	134	×	—	—	—	—	—	—	—	—	—	..... Lungworms present in pneumonic area, 10 x 15 x 15 cm. .....

\* For explanation of symbols see footnote of table 2A.



*Discussion of the Results of Subcutaneous Vaccination and Feeding Exposure.*—A general review of tables 2A and 2B, 3A and 3B will disclose the fact that not a single pig was entirely without macroscopic lesions of tuberculosis upon autopsy regardless of age vaccinated, interval between vaccination and exposure, length of exposure, or length of time between exposure and autopsy. It is also evident from a study of the tables that there was no marked difference in the character and extent of the lesions whether or not the pigs received 10, 20, or 30 feedings. Direct comparison of the lesions of vaccinated pigs 1 to 14 and controls 101 to 114 in tables 2A and 2B, with reference to the interval between first exposure and autopsy, favors the vaccinated pigs slightly in the extent and distribution of the lesions. However, if no attention is given to the relation of this time interval the results show no differences in an equal number of vaccinated and controls. It should be noted that all swine represented in this table were of the same age and that the vaccinated were treated at from 3 to 5 days of age and also protected from exposure from 27 to 93 days thereafter. This procedure fulfilled the recommendations of Calmette and Guérin<sup>5</sup> regarding calves. Tables 3A and 3B represent the results on swine that for the most part were much older when vaccinated and also the group that had a longer exposure period. No significant differences in the extent of tuberculosis could be detected between the vaccinated and the controls although the extent of lesions in the two groups was not as marked as in the younger pigs shown in tables 2A and 2B. Age may have been the factor that limited the amount of infection in this group, since each pig received two or three times the number of feedings of tuberculous material as did those in tables 2A and 2B.

The presence of a vaccination lesion at the site of injection of BCG at the time of, and throughout the exposure period, apparently had no "premunizing"<sup>5</sup> effect. Observations of the vaccination nodule in pigs Nos. 6-14 (table 2A) were made for the first 72 days, which was 21 days before exposure, and at that time the nodules in the flanks had slightly decreased in size from the maximum size, which occurred between 28 and 42 days. Abscess formation with spontaneous evacuation occurred in only one flank of one pig. The maximum size of the vaccination lesions when measured with calipers through the skin of the flank was approximately  $2 \times 4$  cm. The character of the nodules was hard and nonsensitive. At autopsy, as shown in table 2A, the lesions had either disap-

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<sup>5</sup> According to Calmette, the word "premunition" was first proposed in 1924 by Sergent and Donatien to designate a condition of protective latent infection, such as exists in certain protozoan diseases, particularly bovine piroplasmiasis.

peared or were reduced to a very small size. When incised, a thin connective tissue capsule enclosed a soft caseous pus of slightly greenish white color. Acid-fast organisms were always present in the pus.

#### INTRAMUSCULAR VACCINATION AND INFECTION EXPOSURES BY FEEDING MILK FROM A TUBERCULOUS UDDER

Swine shown in table 4 were fed milk from an aged tuberculous cow (No. 600) in which tuberculosis of the udder was present. At the time that the feeding tests began on February 5, 1928, the right hind and the left fore quarters were indurated and about twice normal size. The secretion from these quarters was a clear amber fluid, containing a small amount of gray flocculent material which settled quickly on standing. The left fore quarter showed about one acid-fast organism to every five fields. The other quarters were negative for acid-fast organisms by smears. This cow was destroyed on March 6, 1928, which was 4 days after the feeding period closed. Extensive tuberculosis of the lungs, the costal pleura, and the peritoneum, existed together with marked tuberculosis of right hind and left fore quarters. Material from the latter quarter showed only two typical acid-fast organisms after 50 fields were examined and one clump of three acid-fast upon examination of 26 more fields.

During the time that the pigs were being fed milk from cow No. 600 she was being milked night and morning and at each milking gave from 200 to 300 cc of apparently normal milk from the right front teat. From each of the other three teats it was possible to withdraw 20 to 50 cc of abnormal secretion. An occasional microscopic examination of the milk from each of the teats was made during the feeding period and acid-fast bacilli were always demonstrated microscopically in the secretion from the left fore quarter. They were never found in large numbers since it was always necessary to search several fields of a thick smear before any could be found. The entire secretion from the four teats was mixed with a 10 per cent solution of dry skim milk and fed in equal amounts, care being taken to observe that each animal drank the entire portion allotted to it without spilling.

*Discussion of Results of Intramuscular Vaccination and Infection Exposure by Milk from Tuberculous Udder.*—Reference to table 4 shows two groups of pigs, one of 4 and one of 7, in which very little tuberculosis was produced by the particular type of infection. The groups differ in that the first 4 pigs were just past seven months of age

TABLE 4  
RESULTS OF INTRAMUSCULAR VACCINATION\* AND INFECTION EXPOSURE BY FEEDING MILK FROM COW WITH TUBERCULOUS UDDER

Pig No.	Amount of BCG vaccine	Interval in days			Size of vaccination nodule in mm	Tuberculous lesions†								Acid-fast bacilli in smears‡	Guinea pig inoculation	Remarks		
		Birth to vaccination	Vaccination to first exposure	First infecting feeding to autopsy		Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver				Other lesions	
23	100 mg	186	28	74	50 x 50 x 20	-	-	-	-	-	-	-	-	-	-	+	-	Right anterior lobe of lung showed glassy nodule 2 mm in diameter. Guinea pig did not develop tuberculosis from injection. Acid-fast bacilli were present in nodule.
24	100 mg	186	28	74	30 x 20 x 20	-	-	-	-	-	-	-	-	-	-	-	-	No visible lesions. Guinea pigs negative from injection of tissues.
123	Control	.....	.....	74	.....	-	-	-	-	-	-	-	-	-	-	-	-	No visible lesions. Guinea pigs negative from injection of tissues.
124	Control	.....	.....	74	.....	-	-	-	-	-	-	-	-	-	-	-	-	No visible lesions. Guinea pigs negative from injection of tissues.
25	100 mg	4	34	74	-	-	/	-	-	-	-	-	-	-	-	+	+	Thoracic lymph nodes enlarged 4X to 6X with caseous foci, and about 54 nodules 3-5 mm in diameter, scattered throughout the lungs.
26	100 mg	4	34	74	5 x 5	-	/	-	-	-	-	-	-	-	-	+	+	Right and left bronchial lymph nodes enlarged and caseous. One nodule 4 mm diameter and 4 nodules 2-3 mm in lungs.
27	100 mg	4	34	74	-	-	-	-	-	-	-	-	-	-	-	-	+	Guinea pig No. 580 injected with cervical and mesenteric nodes developed generalized tuberculosis.
125	Control	.....	.....	74	.....	-	/	-	-	-	-	-	-	-	-	+	+	Bronchial lymph nodes enlarged 5X, and caseous. Lungs showed 9 tubercules 2-10 mm in diameter.
126	Control	.....	.....	74	.....	-	X	-	-	-	-	-	-	-	-	-	+	Right and left bronchial lymph nodes enlarged and 50 per cent caseous.
127	Control	.....	.....	36	.....	-	/	-	-	-	-	-	-	-	-	+	+	Accidentally killed.
128	Control	.....	.....	36	.....	-	/	-	-	-	-	-	-	-	-	+	+	Accidentally killed.

\* Nos. 23 and 24 received 100 mg BCG in muscles of forearm; Nos. 25, 26, and 27 received 100 mg in muscles of the thigh.

† For explanation of symbols see footnote of table 2A.

‡ Does not include smears from vaccination lesion.

when exposed and the two vaccinated had BCG injected into the muscles of the forearm; whereas the last seven were 38 days old when exposed and the three vaccinated had BCG injected into the muscles of the thigh. Neither controls nor vaccinated acquired tuberculosis in the first group, with the possible exception of No. 23 in which one lobe of the lung showed a 2 mm nodule that contained a few acid-fast bacilli but which did not infect guinea pigs. Since the second group all showed slight macroscopic lesions (with the exception of pig No. 27, whose apparently normal cervical and mesenteric glands, however, produced lesions in one guinea pig), it is to be concluded that (1) infection from the udder was not massive and (2) group one showed greater resistance because of age. It may be further stated that the pigs in the first group were litter mates, and those in group two were litter mates, but the groups came from different sources.

### SUBCUTANEOUS VACCINATION AND INFECTION BY INTRAVENOUS INJECTION OF VIRULENT CULTURE

In this group are included 6 vaccinated and 5 controls, all of which were 3½ months old at the beginning of the experiment. Two were given 100 mg of BCG, two, 250 mg, and two, 500 mg, subcutaneously in the flank. Nos. 29 and 33 (table 5), which received 500 mg, were vaccinated by injecting 250 mg into each flank. Sixty days after vaccination the vaccinated and the controls were injected intravenously through an ear vein with 2 mg of bovine tuberculosis culture 271, which had grown upon Duval's medium for 28 days. The history of this culture is as follows: On September 1, 1926, guinea pig 132 was injected intramuscularly with liquid obtained by grinding together tuberculous lesions from 17 cows killed at an abattoir. Guinea pig 132 died of generalized tuberculosis on September 19, 1926, and a portion of its spleen was inoculated into guinea pig No. 210, which died of generalized tuberculosis on November 5, 1926. The spleen of No. 210 was inoculated into guinea pig No. 271, which died of generalized tuberculosis on December 22, 1926. Cultures of tubercle bacilli were obtained from the tissues. These proved to be highly virulent for guinea pigs, rabbits, cattle, and swine.

*Discussion of Results of Subcutaneous Vaccination and Intravenous Infection.*—Observation of table 5 does not show marked differences in the extent and distribution of tuberculous lesions between vaccinated and control swine though there is some evidence in favor of the vacci-

TABLE 5  
RESULTS OF SUBCUTANEOUS VACCINATION AND INFECTION EXPOSURE BY INTRAVENOUS INJECTION OF 2 MG OF VIRULENT CULTURE OF TUBERCULOSIS

Pig No.	Amount of BCG vaccine	Interval in days			Size of vaccination nodule	Tuberculous lesions*							Acid-fast bacilli in smears†	Guinea pig inoculation	Remarks
		Birth to vaccination	Vaccination to injection	Injection to autopsy		Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
28	100 mg	105	60	237	?	/	X	/	/	X	X	/	/	+	.....
29	500 mg	105	60	241	1 cm	/	X	/	/	X	X	/	/	+	.....
30	250 mg	105	60	241	1.5 cm	/	X	/	/	X	X	/	/	+	.....
31	100 mg	105	60	243	3 x 5 mm	/	X	/	/	X	X	/	/	+	.....
32	250 mg	105	60	243	2 x 3 cm	/	X	/	/	X	X	/	/	+	.....
33	500 mg	105	60	243	2 x 1 cm and 4 x 3 cm	/	X	/	/	X	X	/	/	+	.....
129	Control	.....	.....	237	.....	/	X	/	/	X	X	/	/	+	Excellent condition when killed. Only partial intravenous injection in each ear; remainder subcutaneous or intradermal.
130	Control	.....	.....	209	.....	X	X	X	/	X	X	X	/	+	Killed because of advanced tuberculosis. Costal pleura tuberculous.
131	Control	.....	.....	209	.....	X	X	/	/	X	X	/	/	+	Killed because of advanced tuberculosis. Partial subcutaneous ear injection; 4 nodules 2 mm in diameter. Costal pleura tuberculous.
132	Control	.....	.....	192	.....	/	X	/	/	X	X	/	/	+	Killed because of advanced tuberculosis. Costal pleura tuberculous.
133	Control	.....	.....	133	.....	/	/	/	/	/	X	/	/	0‡	Died and was too decomposed at autopsy for guinea pig injections. Tuberculous pneumonia caused death.

\* For explanation of symbols see footnote of table 2A

† Does not include smears from vaccination lesion.

‡ "0" indicates that no guinea pigs were injected.

nated. However, there was a marked difference in the clinical effect upon the two groups. All of the vaccinated were in excellent physical condition when autopsied, whereas 4 of the 5 controls were necessarily killed or had died from emaciation and advanced tuberculosis, in 209 days. On the basis of reported results of experiments by the writers, and by Calmette and Guérin, with calves vaccinated and infected by these methods, greater protection than was obtained was expected in this experiment. The larger doses of BCG did not influence the results. It is evident also that the bovine tuberculosis culture injected intravenously was not as virulent for pigs as for calves. In a previous experiment with 2 control calves, both died at 27 and 32 days respectively, of miliary tuberculosis, while the first control hog died at 133 days. None of the swine in this group would have been passed for food under official inspection.

#### INTRAVENOUS VACCINATION AND INFECTION EXPOSURE BY FEEDING

Only 2 vaccinated and 3 controls are reported in this trial. Two other vaccinated and 1 control became sick from an intercurrent disease and their records are not included. Five infecting feedings were given to this group over a period of 12 days, yet these were sufficient to establish moderate lesions in pigs over five months of age when exposed. One mg of BCG was administered intravenously into an ear vein, and 62 days thereafter the first exposure took place.

*Discussion of the Results of Intravenous Vaccination and Feeding Infection.*—The 2 vaccinated and the 2 controls of the same age, exposed and killed at exactly the same periods, and the 1 control killed only six days later, showed no differences upon which any conclusion as to the effects of this method of vaccination can be drawn, except that no resistance against the infection was evident; in fact vaccinated pig No. 35 had slightly more extensive lesions than any of the controls. Massive infecting doses over a short period of time were used in this group.

#### INTRADERMAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

Two separate groups with 2 vaccinated hogs in each, were included in this experiment (tables 7 and 8). The 2 vaccinated pigs shown in table 7 received 50 mg of BCG in two doses of 25 mg each, 3 days apart. Those in table 8 received the same amount in one dose distributed in three

TABLE 6  
RESULTS OF INTRAVENOUS VACCINATION AND INFECTION EXPOSURE BY FIVE FINDINGS OF TUBERCULOUS TISSUES

Pig No.	Amount of BCG intra-venously	Interval in days		Tuberculous lesions*							Acid-fast bacilli in smears	Guinea pig inoculation	Remarks
		Birth to intra-venous vaccination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver	
34	1 mg	93	62	165	/	/	/	/	/	/	/	/	Pancreas contained many foci, 2-5 mm in diameter
35	1 mg	93	62	165	x	x	x	/	x	/	/	/	
134	Control			165	x	x	x	/	/	/	/	/	
135	Control			165	x	x	x	/	/	/	/	/	
136	Control			171	x	/	/	/	/	/	/	/	

\* For explanation of symbols see footnote of table 2A.

different areas. In each case the 50 mg of vaccine was prepared in 5 cc of diluent for injection. The intradermal injections were made along the ventral surface of the abdomen. Exposure of those recorded in table 7 was by means of the ground lesions (prepared as previously described) from control tuberculous hogs, Nos. 112, 113, and 114, and from guinea pigs previously injected with swine lesions. The material for each of the four feedings showed acid-fast bacilli in smears, and guinea pigs injected had generalized tuberculosis when killed. Exposure of those in table 8 was entirely from bovine lesions obtained at a slaughter house, but was not the same as used in some of the other experiments with feeding exposure.

*Discussion of Results of Intradermal Vaccination and Feeding Exposure.*—The results shown in table 7 are difficult to explain except on the basis that the infecting material was not very virulent. This is indicated by the fact that the 2 vaccinated intradermally, and 4 of the 5 controls, showed no macroscopic lesions; and guinea pigs injected with apparently normal tissues did not develop tuberculosis, except in the case of vaccinated No. 37 whose gastrohepatic lymph nodes produced tuberculosis in two guinea pigs. Tissues from head glands, bronchial lymph glands, and lungs failed to infect guinea pigs. Contradicting the supposition of nonvirulence are the animals exposed to the same material, as shown in table 9: control 140 which developed extensive tuberculosis, and vaccinated pigs 40, 41, 42, 43, and 45 which had mild lesions. The results shown in table 8 are not significant, though the 3 controls had a slightly wider distribution of lesions than did the 2 vaccinated ones.

#### ORAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

In this experiment (see table 9) six pigs received BCG by the mouth. Three were given a total of 3 grams (weight after blotting with filter paper) by administering 1 gram every other day for three feedings. The other 3 were given twice this amount at the same periods. The vaccine was prepared so that each 5 cc. of diluent contained 1 gram. This was then deposited on the fauces by means of a syringe. The first exposure of the 6 treated and the five controls took place 60 days after oral vaccination. All of the pigs were 7 to 8 days of age when the experiment started. The infecting material was the same as that used on pigs in table 7 and as has been stated under discussion of the latter group apparently was not very virulent for the swine.



TABLE 7  
RESULTS OF INTRADERMAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

Fig No.	Number of feedings	Interval in days				Tuberculous lesions†								Guinea pig inoculations	Remarks
		Amount of BCG intradermally*	Birth to intradermal vaccination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
36	4	50 mg	7	60	200	-	-	-	-	-	-	-	-	-	No lesions except slight verminous pneumonia. Guinea pigs injected with various lymph nodes remained well.
37	4	50 mg	8	60	194	-	-	-	-	-	-	-	-	+	No lesions. Guinea pigs injected with gastrohepatic and mesenteric nodes developed tuberculosis.
140	Control	.....	.....	.....	194	-	/	/	-	X	/	X	X	+	The spleen was a mass of tubercles, 1.5 cm. caseous. Liver thickly covered on all surfaces with foci, 2-3 mm.
141	Control	.....	.....	.....	194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with cervical, bronchial, and mesenteric nodes remained well.
142	Control	.....	.....	.....	186	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various nodes remained well.
143	Control	.....	.....	.....	194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various nodes remained well.
144	Control	.....	.....	.....	194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various nodes remained well.

\* Pigs received 50 mg in two doses of 25 mg three days apart.

† For explanation of symbols see footnote, table 2A.

‡ Does not include smears from intradermal lesions.

TABLE 8  
RESULTS OF INTRADERMAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

Pig No.	Number of feed-ings	Interval in days				Tuberculous lesions†							Acid-fast bacilli in smears‡	Guinea pig inoculations	Remarks
		Amount of BCG intra-dermally*	Birth to intra-dermal vac-cination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Meenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
38	5	50 mg	93	62	165	X	X	X	/	/	/	/	/	+	Fibrinous pleurisy present.
39	5	50 mg	93	62	171	X	X	X	/	/	/	/	/	+	.....
137	5	Control	.....	.....	165	X	X	X	/	/	/	/	/	+	.....
138	5	Control	.....	.....	165	X	X	X	/	/	/	/	/	+	.....
139	5	Control	.....	.....	171	X	X	X	/	/	/	/	/	+	Pancreas contained many foci, 2-5 mm in diameter.

\* Pigs received 50 mg in one dose in three different areas.

† For explanation of symbols see footnote, table 2A.

‡ Does not include smears from intradermal lesions.

TABLE 9  
RESULTS OF ORAL VACCINATION AND INFECTION EXPOSURE BY FOUR FEEDINGS OF TUBERCULOUS TISSUES

Pig No.	Total amount of BCG vaccine by mouth *	Interval in days			Tuberculous lesions†							Acid-fast bacilli in smears	Guinea pig inoculations	Remarks
		Birth to oral vaccination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
40	3 gm	8	60	200	/	-	-	-	-	/	-	-	+	Guinea pigs injected with cervical lesions, and with normal-appearing bronchial and gastrohepatic lymph nodes, developed tuberculosis.
41	3 gm	8	60	200	/	-	-	-	-	-	-	-	+	Guinea pig injected with a cecal caseous focus died of tuberculosis.
42	3 gm	7	60	185	-	-	-	/	-	-	-	-	+	Three pigs injected with other tissues remained normal.
43	6 gm	8	60	200	×	×	-	-	/	?	-	-	+	Lung showed area of pleurisy and hepatization. Guinea pigs injected with bronchial and gastrohepatic lymph nodes developed tuberculosis.
44	6 gm	7	60	185	-	-	-	-	-	-	-	-	-	Cephalic lobe of right lung showed gray hepatization area 8 x 15 cm. Guinea pig died. Three guinea pigs injected with other tissues remained well.
45	6 gm	7	60	200	/	/	-	-	-	/	-	-	+	The guinea pig injected with bronchial lesions developed tuberculosis; the others remained well.
140	Control	.....	.....	194	/	/	/	-	×	/	×	×	+	The spleen was a mass of tubercles, 1-5 cm caseous. Liver thickly covered on all surfaces with foci, 2-3 mm.
141	Control	.....	.....	194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with cervical, bronchial, and mesenteric lymph nodes, remained well.
142	Control	.....	.....	186	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various lymph nodes remained well.
143	Control	.....	.....	194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various lymph nodes remained well.
144	Control	.....	.....	194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various lymph nodes remained well.

\* Doses of BCG vaccine by mouth were given every other day for 3 days until the total quantity of 3 gm and 6 gm, respectively, was administered.

† For explanation of symbols see footnote, table 2A.

*Discussion of Results of Oral Vaccination and Feeding Exposure.*—An interpretation of the data from this experiment is not possible in the light of results on pigs, shown in table 9, which received the same exposure. All the pigs in both groups were from the same source and of the same age, yet 4 out of 5 controls showed no lesions, and only 2 out of 8 that were vaccinated were entirely free of tuberculous changes. If the same principles of interpretation that have been applied to the preceding tables of results are applied to the records in table 9 they would suggest a slightly increased susceptibility following the oral administration of BCG.

#### OBSERVATIONS OF THE EFFECT OF BCG ON SWINE NOT EXPOSED TO TUBERCULOUS INFECTION

As an additional control on the effects of the vaccine some of the swine treated in various ways with BCG were retained in an environment kept as free as possible from virulent tubercle bacilli. Following is a list of these trials:

1. Fifteen swine at various ages from 1 day to 6 months were vaccinated subcutaneously or intramuscularly with 100-mg doses and held for varying lengths of time from 40 days to 14 months after which they were slaughtered and examined for tuberculous lesions. Small abscesses containing acid-fast bacilli nonpathogenic for guinea pigs were present at the point of injection in most of the swine, but no other evidence of tuberculosis was found. In addition, a boar aged six months was injected subcutaneously and intramuscularly with 80 cc of a suspension containing 800 mg of BCG. The injections were made in small amounts in various parts of the body. On slaughter after 13 months no lesions resembling tuberculosis could be found nor any trace of local lesions at the points of injection.

2. Two pigs, one aged 8 weeks and one aged 7 months, were each fed 7 grams of BCG in milk. On slaughter 6 months later no lesions resembling tuberculosis were found.

3. Eight pigs, aged 6 months, were each injected intravenously with 100 mg BCG suspended in physiological sodium chloride solution. They remained healthy in appearance. Thirty days after injection, 2 were slaughtered and their tissues were apparently normal throughout except that on histological examination the lungs were seen to be studded with lesions attributable to BCG which had lodged in the pulmonary capillaries. Four more were slaughtered 43 days after injection. On macroscopic inspection these were also apparently free from tubercu-

lous lesions but on close scrutiny the lungs were seen to be studded throughout with glass-like nodules, pin point to 0.5 mm in size. A histological study showed these to be minute tubercles containing acid-fast bacilli.

The two other swine were retained for nine months and on slaughter no lesions resembling tuberculosis were found. A close macroscopic scrutiny of the lungs showed nothing abnormal. A histological examination was not made.

In order to test the possibility of producing clinical symptoms by a massive intravenous injection, a gilt aged three months was given by the ear vein 50 cc of physiological sodium chloride solution in which 500 mg of BCG were suspended. The day after the injection, partial paralysis of the rear limbs developed, from which the animal slowly recovered although it was still slightly lame on slaughter 13 weeks after the BCG was injected. At autopsy all tissues were seen to be apparently normal except the lungs which were studded with white nodules up to 0.5 mm in size. A histological study showed these to be necrotic foci containing acid-fast bacilli and surrounded by a wall of epithelioid cells. Guinea pigs inoculated with lung and lymphatic tissue of this gilt remained normal.

The dates on which these trials were made ranged from May, 1926, to September, 1931. The cultures used ranged from the second to the seventieth transplant made following its receipt from Calmette. During this time 328 head of cattle, 356 guinea pigs, and 15 rabbits, as well as the 85 swine mentioned in this paper, were vaccinated. No indication of change in virulence or morphology has been observed in the cultures during these five years. The culture is relatively nonpathogenic for swine. It is capable of causing small lesions at the point of inoculation which soon become surrounded by firm white fibrous connective tissue and are eventually reduced in size or completely disappear.

## CONCLUSIONS

One injection of Calmette-Guérin culture (BCG) by subcutaneous, intramuscular, intradermal, or intravenous routes, or three treatments by mouth, failed to give swine sufficient protection against feeding and intravenous infection to prevent generalized tuberculosis.

Slight resistance as compared to the controls was shown by certain groups vaccinated subcutaneously and infected by feeding.

Those vaccinated subcutaneously showed slightly greater resistance against intravenous infection than against feeding exposure, as measured by clinical evidence.

Those vaccinated by mouth showed a slightly greater susceptibility to feeding infection than the control to feeding infection.

Swine over six months of age apparently had more natural resistance to feeding exposure than younger pigs.

No important differences were noted in the extent of tuberculosis produced from 4, 5, 10, 20, and 30 feeding exposures.

The BCG culture used was capable of producing small lesions in swine which eventually healed, at the points where the bacilli lodged after injection. No spread from these primary lesions was observed.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

DECEMBER, 1932

No. 7

## DETERRENT EFFECT OF ARTIFICIAL LIGHT ON THE CODLING MOTH<sup>1, 2</sup>

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The results of a codling-moth light experiment conducted during the summer of 1928 showed that the percentage of worminess in apples from trees in an artificially lighted area was less than that of apples from trees in an unlighted area: it was, therefore, concluded that light has a tendency to deter the codling moth in its egg-laying habits.<sup>4</sup> When fruit of the same variety was compared at the end of the test, it was found that 21.0 per cent of the apples on check trees outside the test plot were moth attacked, while only 14.5 per cent of the apples inside the test plot were so affected.

### CONDITIONS OF THE EXPERIMENT

For the purpose of that experiment, six 500-watt lamps were used during the evening hours from about an hour before sunset to about an hour after sunset, an illumination time of about 2 hours, during the period from May 1 to June 30. The peak of the codling-moth flight occurs from about 20 minutes before sunset to about 20 minutes after, hence the hours chosen for artificial illumination. A block of 15 trees was used.

<sup>1</sup> Received for publication July 2, 1932.

<sup>2</sup> This article is a contribution of the Division of Entomology and Parasitology, and the California Committee on the Relation of Electricity to Agriculture. It is the eighth of a series planned to report the results of investigations conducted jointly by the Agricultural Experiment Station, College of Agriculture, and the California Committee on the Relation of Electricity to Agriculture. The Committee represents the agricultural and electrical industries in California that are working together, in cooperation with similar committees in other states, for the purpose of making available reliable information concerning the use of electricity on the farm.

<sup>3</sup> Professor of Parasitology and Entomologist in the Experiment Station.

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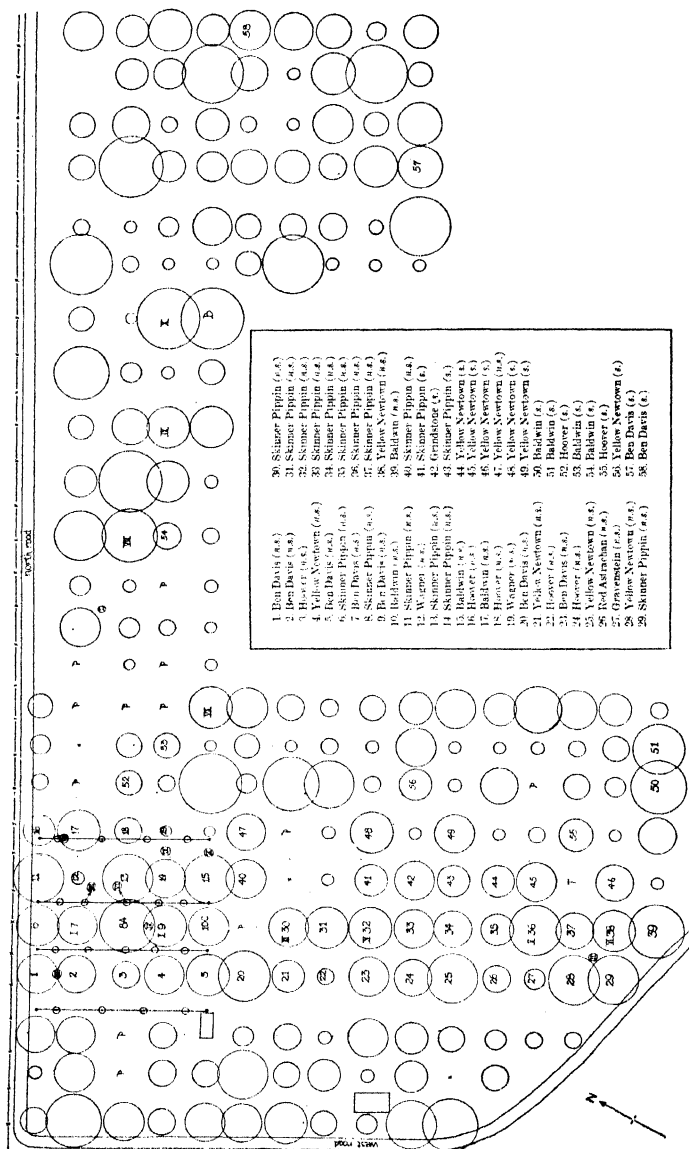


Fig. 1. Location of lights; light-intensity stations (I - X in circles); thermograph stations (A, B, C); and bait-pan stations (I - X) in the orchard. The relative sizes of trees are indicated by the sizes of the circles. A key to the varieties of trees is given on the map; s. indicates sprayed trees, and n.s. nonsprayed trees.

For the 1929 test the same plot of trees was used (fig. 1), but the number of 500-watt lamps was increased to eighteen and the period of illumination extended through the entire season, namely, from April 24 to October 24. During this entire time the lights were on every evening from 2 to 4 hours. All apples, including thinnings and windfalls, were examined for worminess. A total of 217,975 apples were examined from 51 trees, selected to permit comparison of fruit from illuminated and nonilluminated trees, and from sprayed and unsprayed trees.

The lamps (Mazda, 500-watt) were suspended over the tops of the trees from wires strung between poles as shown in figure 2. Twelve-inch white porcelain reflectors were used. The position of the lamps with reference to the tree tops depended, of course, upon the size of the trees.



Fig. 2. Arrangement of lights suspended over the trees.

The light-intensity readings were taken by means of a MacBeth illuminometer. The automatic switch used for controlling the lights was a two-pole, 115/230-volt Sauter time switch furnished with astronomic dial.

Ten stations were selected for light-intensity readings as shown in figure 1. Both temperature and humidity records were kept, as well as records on sunset time, weather conditions, and observed moth flights. Daily reports showing the above data were made in duplicate, and from this material a consolidated table was prepared. This consolidated table with supporting data is on deposit in the office of the California Agricultural Experiment Station at Berkeley, where it may be consulted by those interested. For the purpose of this report only an extract from the general table is here included to show characteristic data. (See table 1.)

TABLE 1.—CLIMATIC DATA, LIGHT-INTENSITY READINGS AND BAIT-PAN CATCHES.

Date	Daily temperature				Sunset		Humidity		Light				
	Minimum		Maximum		Time P.M.	Weather	Time Hum. P.M.	per cent	On		Off Time Temp. P.M.		
	Time A.M.	Temp. °F.	Time P.M.	Temp. °F.					Time P.M.	Temp. °F.			
1020 May 22	6	48	2	71	7:12	54	Windy, cloudy	5:30 6:30 8:30	88 88 94	6:15	60	9:00	40
23	4	44	3	79	7:13	60	Calm, full moon	5:30 6:30 8:30	76 90 94	6:15	69	9:00	52
24	6	43	2	87	7:14	68	Calm	5:30 6:30 8:30	66 88 79	6:15	74	9:00	57
25	6	39	2	79	7:15	63	Breeze, clear	5:30 6:30 8:30	64 66 72	6:30	67	9:15	57
26	6	50	3	76	7:15	63	Breeze, clear	5:30 6:30 8:30	70 80 85	6:30	66	9:15	55
27	6	38	2	83	7:16	64	Calm, clear, sunny	5:30 6:30 8:30	30 36 56	6:30	70	9:15	51
28	6	37	1	80	7:17	60	Calm, no moon	5:30 6:30 8:30	52 57 81	6:30	66	9:15	40
29	5	38	2	72	7:18	52	Breeze, foggy	5:30 6:30 8:30	69 77 89	6:30	57	9:15	46

DURING THE TWO PEAK FLIGHTS OF THE CODLING MOTH; SEBASTOPOL, CALIFORNIA, 1929

Light-intensity readings in foot-candles				Number of moths in each bait trap															
Time	P. M.	Stations outside lighted area				Stations inside lighted area		Number of moths in each bait trap										Total	
		No. 1	No. 4	No. 6	No. 8	No. 10	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10			
6:30			969.0	424.0		432.0	338.0												
7:00			69.0	97.0		96.0	39.0												7
7:30			6.5	38.0		33.0	2.6												
8:30			5.4	33.0		31.0	2.4												
6:00			1,574.0	338.0		1,738.0	362.0												31
7:00			119.0	115.0		125.0	56.0												
8:00			5.2	31.0		30.0	2.5												
9:00			5.5	33.0		31.0	2.6												
6:00			1,574.0	331.0		1,737.0	302.0												127
7:00			74.0	113.0		121.0	55.0												
8:00			5.1	31.0		29.0	2.2												
9:00			5.5	32.0		29.0	2.4												
6:00			1,337.0	456.0		1,405.0	449.0												97
7:00			110.0	120.0		120.0	57.0												
8:00			6.3	34.0		32.0	2.5												
9:00			5.5	32.4		27.8	2.2												
6:30			125.0	167.0		213.0	189.0												138
7:30			6.4	9.0		31.0	14.0												
8:30			4.4	29.7		20.9	2.4												
9:00			5.0	31.5		29.7	2.6												
6:00			1,456.0	377.0		1,405.0	401.0												139
7:00			102.0	138.0		142.0	79.6												
8:00			4.8	29.7		24.0	2.3												
9:00			4.9	30.6		27.8	2.5												
6:00			1,337.0	315.0		1,574.0	354.0												24
7:00			118.0	177.0		119.6	59.0												
8:00			4.4	29.6		25.9	2.5												
9:00			5.1	31.5		28.7	2.7												
6:00			1,359.0	362.0		1,385.0	377.0												6
7:00			101.0	156.9		110.0	110.0												
8:00			5.2	30.6		29.6	2.4												
9:00			5.3	33.7		31.5	2.5												

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TABLE I—CLIMATIC DATA, LIGHT INTENSITY READINGS AND BAIT PAN CATCHES DURING

Date	Daily temperature					Sunset		Lagbia			
	Minimum		Maximum		Time P M	Temp ° F	Weather	On		Off	
	Time A M	Temp ° F	Time P M	Temp ° F				Time P M	Temp ° F	Time P M	Temp ° F
1929 June 18	6	46	1	79	7 30	64	Calm, clear, moon	6 30	70	9 30	86
19	6	49	1	93	7 31	70	Calm clear sul- try all day	6 30	79	9 30	88
20	6	49	4	98	7 31	83	Calm sultry	6 30	89	10 00	70
21	6	50	2	102	7 32	79	Calm sultry full moon at 8 P M	6 45	86	10 00	69
22	6	54	3	96	7 32	72	Calm	6 45	70	10 00	60
23	6	54	3	100	7 32	72	Calm	6 45	78	10 00	83

THE TWO PEAK FLIGHTS OF THE CODLING MOTH; SEBASTOPOL, CALIFORNIA, 1929 ----(Concluded)

## OBSERVATIONS ON EGG-LAYING HABITS

An artificial light intensity sufficient to deter the moths from egg-laying had been hoped for but this was achieved only to a limited extent. With the trees flooded with light every evening for the entire season it was possible to make many interesting supplementary observations relative to the habits of the codling moth, such as actual egg deposition, mating, flight, etc. These supplementary observations are recorded in a paper by Borden.<sup>5</sup>

A few of these observations are, however, pertinent to this report. Thus the records show that the flight of the moths may extend over a period of approximately 2 hours when weather conditions are favorable, and that the maximum number in flight is usually to be found from about 20 minutes before sunset to about 20 minutes after. The deposition of eggs took place mainly during this sunset period at temperatures ranging from 60° to 69° F, and when there is almost no movement of the air. Actual egg deposition was observed, and light measurements and other weather observations were made at the time; among these observations are those given in table 2.

TABLE 2  
CODLING MOTH EGG DEPOSITIONS OBSERVED UNDER KNOWN LIGHT, TEMPERATURE,  
AND HUMIDITY CONDITIONS

Date	Conditions when egg was laid				Time of sunset
	Time	Temperature	Light intensity	Humidity	
1929	P.M.	°F	foot-candles	per cent	P.M.
April 28.....	5:45	56	1,298.0	94	6:51
May 2.....	7:45	67	12.0	71	6:55
August 13.....	7:45	65	17.0	70	7:01
August 18.....	7:07	65	10.0	72	6:52
August 18.....	7:15	64	5.8	75	6:52
August 22.....	6:42	69	11.0	68	6:46
August 25.....	6:18	65	40.2	75	6:40
September 10.....	7:15	64	3.3	72	6:18

That the codling moth may lay its eggs even under very high light intensities is borne out by the April 28 observation, when an egg was laid during a light intensity of 1,298 foot-candles. Moths were first observed in the orchard April 24.

<sup>5</sup> Borden, A. D. Some field observations on codling moth behavior. Jour. Econ. Ent. 24(6):1137-1145. 1929.

It is a well-known fact that varieties of apples show a variation in susceptibility to codling-moth attack. This is borne out by the observations recorded in table 3; the worminess in the unsprayed, non-illuminated trees of the Ben Davis variety, for example, was 90.9 per cent, whereas in those of the Yellow Newtown variety it was 55.9 per cent.

TABLE 3

VARIETAL SUSCEPTIBILITY OF APPLES TO CODLING-MOTH ATTACK UNDER ILLUMINATED AND NONILLUMINATED CONDITIONS

Variety	Not sprayed						Sprayed	
	Fully illuminated		Partially illuminated		Not illuminated		Not illuminated	
	Per cent wormy	Tree Nos.	Per cent wormy	Tree Nos.	Per cent wormy	Tree Nos.	Per cent wormy	Tree Nos.
Ben Davis .....	80.9	2, 7, 9	93.6	1, 5, 20	90.9	23	5.4	57, 58
Yellow Newtown ..	47.2	4	59.5	21, 47	55.9	25	9.1	44, 46, 48, 49, 56
Skinner Pippin.....	44.1	8, 13, 14	60.0	6, 11, 30, 40	89.1	31, 32, 33, 34, 35	17.0	41, 43
Hoover.....	35.3	3	56.2	16, 18	67.9	24	3.8	55
Baldwin.....	.....	.....	58.3	10, 15, 17	.....	.....	4.2	50, 51, 54

The data shown in table 3 also support the assumption that artificial illumination as a deterrent had much the same effect on the codling moth with each of the several varieties of apples.

TABLE 4  
PERCENTAGE OF WORMY APPLES FROM ALL TREES USED IN LIGHT EXPERIMENT

Tree No	Variety of apple	Windfalls		Thinned		Picked		Total	
		Number of apples	Per cent wormy	Number of apples	Per cent wormy	Number of apples	Per cent wormy	Number of apples	Per cent wormy
1†	Ben Davis	520	94			655	95	1,175	94
2†	Ben Davis	80	91			97	87	177	88
3†	Hoover	247	92	925	6	2,025	42	3,197	35
4†	Yellow Newtown	693	95	1,391	19	2,708	49	4,792	47
5†	Ben Davis	56	96			105	92	161	94
6†	Skinner Pippin	434	93	411	14	1,389	64	2,234	61
7†	Ben Davis	602	84			2,133	79	2,735	80
8†	Skinner Pippin	810	87	794	10	3,945	55	5,549	53
9†	Ben Davis	681	88			1,624	79	2,305	81
10†	Baldwin	834	94	484	26	1,525	71	2,343	70
11†	Skinner Pippin	128	92			323	88	451	89
12†	Wagner*								
13†	Skinner Pippin	744	89	1,212	4	4,956	40	6,912	38
14†	Skinner Pippin	1,103	85	1,314	9	4,313	41	6,730	42
15†	Baldwin	1,543	94	2,139	18	6,302	63	9,984	58
16†	Hoover	793	93	1,353	19	3,236	55	5,382	51
17†	Baldwin	727	97	1,978	34	2,978	54	5,683	53
18†	Hoover	246	97			415	95	661	96
19†	Wagner*								
20†	Ben Davis	303	89			287	93	590	91
21†	Yellow Newtown	993	98	1,297	31	2,822	59	5,112	59
22†	Hoover*								
23†	Ben Davis	68	88			42	95	110	91
24†	Hoover	278	85	171	12	791	60	1,240	68
25†	Yellow Newtown			2,158	28	5,362	54	9,483	56
26†	Red Astrachan*	1,963	92						
27†	Gravenstein*								
28†	Yellow Newtown	1,570	93	3,730	19	8,100	36	13,400	36
29†	Skinner Pippin	717	92	1,701	13	3,938	38	6,356	38

TABLE 4—(Concluded)

Tree No	Variety of apple	Illumination	Windfalls		Thinned		Picked		Total	
			Number of apples	Per cent wormy	Number of apples	Per cent wormy	Number of apples	Per cent wormy	Number of apples	Per cent wormy
30†	Skinner Pippin.	Partial	1,544	92	879	16	3,257	62	5,680	63
31†	Skinner Pippin	None	624	95	37	19	855	85	1,516	88
32†	Skinner Pippin	None	951	96	25	8	1,369	87	2,345	89
33†	Skinner Pippin	None	648	96			761	87	1,409	91
34†	Skinner Pippin	None	619	95			885	84	1,504	88
35†	Skinner Pippin	None	175	91			241	81	416	85
36†	Skinner Pippin	None	1,266	91	1,011	10	3,237	51	5,514	52
37†	Skinner Pippin	None	600	92	512	19	1,527	56	2,639	57
38†	Yellow Newtown	None	1,416	95	2,742	21	4,926	47	9,084	47
39†	Baldwin	None	2,012	93	2,688	21	7,659	51	12,359	52
40†	Skinner Pippin	Partial	1,276	87	923	8	3,628	55	5,827	54
41†	Skinner Pippin.	None	401	23	109	4	1,420	15	1,930	16
42†	Grudstone*									
43†	Skinner Pippin	None	221	31	189	3	1,177	19	1,587	18
44†	Yellow Newtown	None	144	65	588	5	1,199	13	1,931	14
45†	Yellow Newtown*									
46†	Yellow Newtown	None	134	48	300	9	853	15	1,287	17
47†	Yellow Newtown	Partial	1,075	97	1,600	32	3,080	61	5,755	59
48†	Yellow Newtown	None	540	40	1,213	2	3,892	11	5,635	11
49†	Yellow Newtown	None	265	49	2,562	2	5,507	6	8,274	6
50†	Baldwin	None	750	9	1,896	3	8,413	4	11,059	4
51†	Baldwin	None	272	22	1,726	4	4,253	7	6,251	7
52†	Hoover	None	43	18	512	2	1,132	9	1,687	7
53†	Baldwin	None	294	91	1,999	15	3,134	4	5,427	13
54†	Baldwin	None	337	6	2,152	0 40	3,828	1 2	6,317	1
55†	Hoover	None	191	10	945	0 01	2,237	5	3,373	4
56†	Yellow Newtown	None	161	38	971	2	2,718	6	3,850	6
57†	Ben Davis	None	1,065	4			3,718	3	4,783	4
58†	Ben Davis	None	139	3			3,135	8	3,274	8
	Total		33,296		46,577		138,102		217,975	

\* Counts not made because of small size of trees

† Not sprayed

‡ Sprayed



## RELATION BETWEEN PERCENTAGE OF WORMINESS AND INTENSITY OF LIGHT

Table 4 shows the percentage of worminess in all apples from all trees included in this experiment. Segregating the trees according to position in relation to lights, the percentages given in table 5 may be obtained. (See figure 1 for location of trees in the orchard.)

TABLE 5  
TREES GROUPED ACCORDING TO POSITION IN RELATION TO LIGHT,  
SHOWING PERCENTAGES OF WORMINESS

Spraying	Illumination	Number of trees	Tree Nos.	Worminess
				<i>per cent</i>
Unsprayed	Total.....	8	2, 3, 4, 7, 8, 9, 13, 14	49.7
Unsprayed	Dimly (closely bordering lighted area)	14	1, 5, 6, 10, 11, 15, 16, 17, 18, 20, 21, 30, 40, 47	60.2
Unsprayed	Nonilluminated.....	8	23, 24, 25, 31, 32, 33, 34, 35	71.3
Sprayed*	Nonilluminated.....	15	41, 43, 44, 46, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58	7.4

\* The trees received a calyx spray of 3 pounds acid lead arsenate plus 8 pounds of sulfur to make 100 gallons, and two cover sprays of 3 pounds acid lead arsenate plus 6 pounds of sulfur to make 100 gallons.

Out of the total of 58 trees included in the experiment 45 are accounted for in the percentages given in the table. The following are thrown out of consideration for various reasons: Nos. 12, 19, 22, and 45 because of small size; No. 26, a Red Astrachan, No. 27, a Gravenstein, and No. 42, a Grindstone, which are represented in one group only; also Nos. 28, 29, 36, 37, 38, and 39, all unsprayed and not illuminated, which were located in a distinct swale and hence not ecologically comparable with any other part of the general plot under observation. The percentage of worminess of these thirteen trees was 45.6 per cent.

The highest average artificial light intensity maintained night after night throughout the season at any of the stations was at station No. 6, which was about 3 feet distant from the overhead source of light and averaged about 20 foot-candles. The station was about 9 feet above the ground and within 6 to 7 feet below the tops of the adjacent trees in a location favorable for codling-moth activity. In spite of this intensity trees 8 and 13, the two most affected by it, showed 53.4 per cent and 38 per cent worminess respectively, or an average of 45.2 per cent, which is close to the average of the illuminated group (49.7 per cent). Trees

52 and 53 were adjacent to the light area but were sprayed, giving a percentage of worminess of 11.6.

Using the same variety of apple (viz. Skinner Pippin) and proceeding from a point of high artificial illumination, the following percentages of worminess are of interest; trees Nos. 8, 13, and 14 (all illumi-

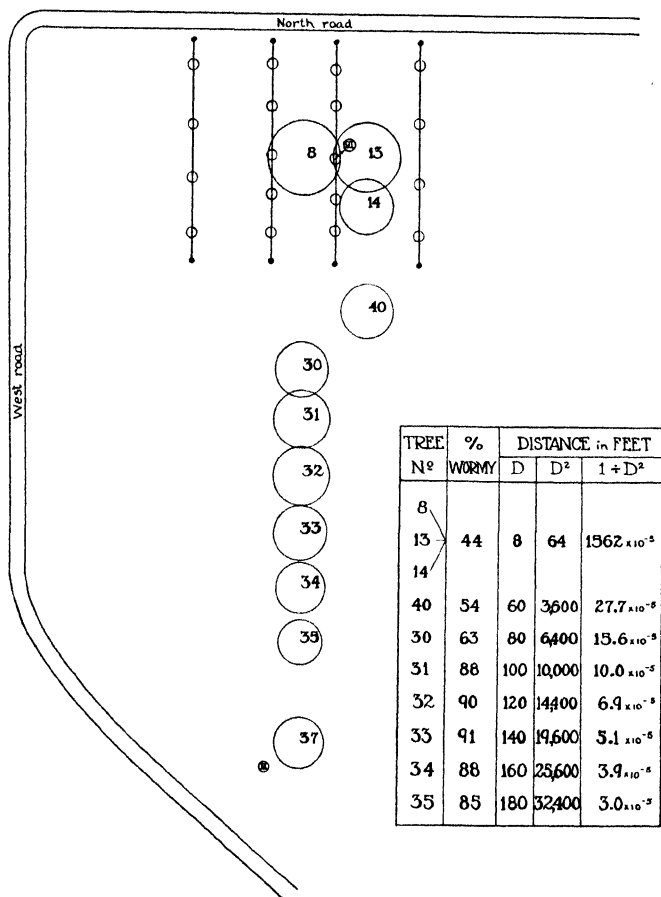


Fig. 3. Location of Skinner Pippin trees in test plot.

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nated) showed 44.1 per cent worminess; Nos. 40 and 30, both in dim light, showed 54.7 and 63.0 per cent respectively; thence away from the light, tree No. 31 showed 88.2 per cent; No. 32, 89.8 per cent; No. 33, 90.9 per cent; No. 34, 88.1 per cent; No. 35, 85.3 per cent. All other factors being equal, one may conclude that differences in light intensity are responsible for this variation in worminess. (See fig. 4.) The aver-

age percentage of worminess of unsprayed trees not subjected to artificial light and in locations far removed from the test plot was estimated at between 85 and 90 per cent, which corresponds very closely to the percentage of worminess for the trees farthest from the source of light, namely Nos. 31, 32, 33, 34, and 35, which show 89.1 per cent worminess.

The curve (fig. 4) showing the percentage of worminess for Skinner Pippins alone is the result of plotting worminess against the reciprocal of the square of the distance from the center of the lighted area. The average distance of the illuminated trees (Nos. 8, 13, and 14) from the

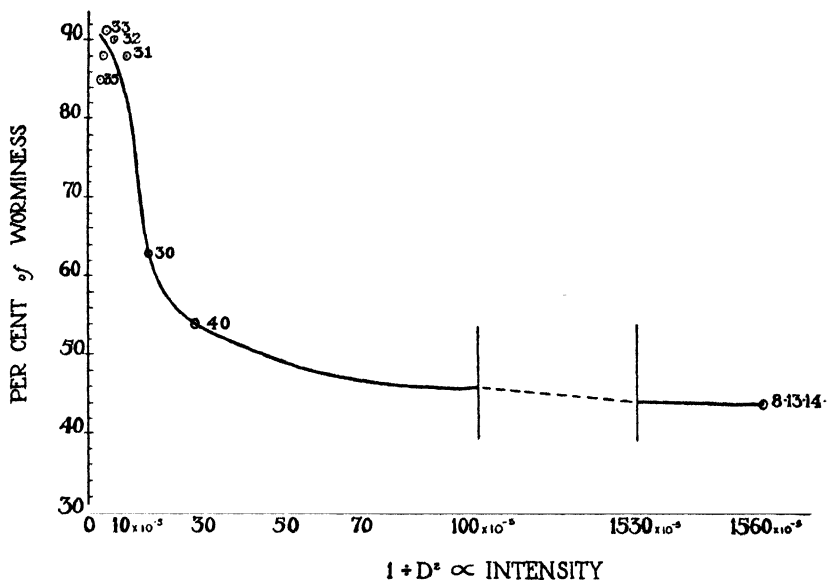


Fig. 4. Relation of the percentage of worminess for Skinner Pippin apples and distance from the source of artificial light. The numbers on the curve represent tree numbers. The curve is the result of plotting worminess against the reciprocal of the square of the distance from the center of the lighted area.

several sources of light in the test plot was estimated at 8 feet, and the other distances are taken from the center of this lighted area. The location of the trees is shown in figure 3.

The intensity of the light is inversely proportional to the square of the distance from the source of light, and figure 4 shows the curve obtained when worminess is plotted against the term  $1/D^2$ , which is closely proportional to the light intensity at every point. This method of measurement is used because of the lack of light stations at the locations of the several trees used for worminess counts. The light-measur-

ing surfaces were held in a horizontal position, therefore such measurements as were actually made did not represent the horizontal variations.

It may be seen that over a very wide range of light intensity as indicated by values of  $1/D^2$  from  $30 \times 10^{-5}$  to  $1,560 \times 10^{-5}$  the percentage of worminess is practically constant, and it is not until the function of  $1/D^2$  falls below  $30 \times 10^{-5}$  that worminess increases abruptly. In spite of the fact that the term  $1/D^2$  for a point source of illumination is used as a theoretical basis, the use of the possibly more acceptable term  $1/D$  for a line source does not alter the conclusions, for the curve, while flatter, indicates the same effect of light.

The object of the experiments, of course, was to produce light conditions artificially in the orchard that would deter the female codling moth in the deposition of her eggs, with the assumption that eggs are laid principally when the light intensity is relatively low and when the weather is favorable. That there is an optimum light condition during which codling-moth activity takes place seems to be the case, and in the 1928 experiments the highest percentage of moth-attacked apples occurred in that portion of the orchard where the light intensity by artificial illumination remained for the evening at from 0.3 to 0.4 foot-candles, i.e., the intensity of natural light about 20 minutes after sunset. To increase that intensity materially and maintain it throughout the daily flight period of the moth would seem to be a fairly easy thing to do, but unfortunately the range of nondeterrent intensity is unquestionably much higher, as shown by the more extensive observations during the season of 1929.

An examination of data included in the consolidated table shows that the natural light intensity (station No. 2) for the period of maximum egg deposition ranges from between 72 and 90 foot-candles 20 minutes before sunset, to between 0.3 and 0.5 foot-candles 20 minutes after sunset, indicating a general range of favorable light intensity of from 0.3 foot-candles to 90 foot-candles during which the codling moth will deposit eggs freely. At sunset there is a natural light intensity averaging 30 foot-candles and ranging from 27 to 52.

Contrasting station No. 2, located outside the artificially illuminated plot, with station No. 6 inside the lighted area, the readings taken at the latter, 20 minutes before sunset show a range of from 92 to 112 foot-candles, and for 20 minutes after, the intensity is from about 11 to 16 foot-candles. At sunset this station shows an average light intensity of 51 foot-candles, ranging from 47 to 54. The variation in light intensity at station No. 2 as compared with station No. 6 is explained by the

fact that the former (No. 2) was situated in a distinct swale, as already explained, so that the sunset hour for that station was advanced.

In spite of the fact that the light intensity in the test plot was increased during the second season, the relative reduction in the percentage of worminess was not greatly altered in view of the fact that there was a very much increased percentage of worminess in general. There was a 31 per cent reduction in worminess in the light area for 1928<sup>6</sup> (illuminated 14.5 per cent wormy; nonilluminated 21 per cent), and a reduction of 30 per cent for 1929 (illuminated 49.7 per cent wormy; and nonilluminated 71.3 per cent). Any efficiency that increased light intensity might have had in the control of the codling moth under normal conditions would no doubt have suffered during a year of unusual moth abundance such as 1929; i.e., 71.3 per cent worminess as compared with 21 per cent in 1928. That 1929 was an unusually heavy codling-moth season is shown by the bait-pan catches. The total number of moths taken from April 30 to July 1, 1928, was 912, while with a similar number of pans similarly located the total number of moths taken for the same period during 1929 was 1,845. The total number of moths taken for the entire season April 26 to September 27 was 2,621. It is of interest to know that the average number of moths taken per tree was larger in the area away from the light, viz., inside the artificially lighted area the average per tree was 244 (pans Nos. 1 and 2), and outside it was 286 per tree (pans Nos. 4, 5, 6, 7, 8, 9, 10).

A field test such as this reveals many questions which can be satisfactorily answered only by further testing in the laboratory. Since the codling moth responds positively to light under laboratory conditions, there remains to be determined the exact range of favorable intensity, together with a more exact determination of the factors which inhibit flight and oviposition. It should be pointed out that since normal maximum egg-laying activity coincides with the rapid decrease of intensity, and inhibition under the conditions of this test appears to be correlated with the maintenance of a constant intensity, i.e., artificial light, the possibility of a change of intensity as in nature as a stimulus to flight and oviposition might be suggested. Since there is some evidence of an occasional sunrise flight this question is further emphasized. Sunrise flights may be rare only because of adverse temperature conditions at that time.

There are also many questions relating to the quality of the light, particularly to the intensity of a specific region of the spectrum. The

<sup>6</sup> Herms, W. B. A field test of the effect of artificial light on the behavior of the codling moth, *Carpocapsa pomonella* (Linn.). Jour. Econ. Ent. 22(1):78-88. 1929.

time of day when flights occur emphasizes the importance of this matter. Laboratory tests concerning intensity, quality, and constancy of light are in progress, and will be the subject of a later paper.

It is freely conceded that the real test of the efficacy of light in combating the codling moth would come if an entire large area were to be illuminated. That moths prevented from entering the area under effective illumination, as involved in the test here reported, might go elsewhere to more attractive darker areas to deposit their eggs is quite probable. Likewise it is unlikely that moths which succeeded in entering the lighted area in spite of the repellent effects of the light would be deterred from laying their eggs. The reported reduction in worminess is believed to be because there were fewer moths in the illuminated area.

## CONCLUSIONS

1. The total number of apples (from 51 trees) examined for worminess was 217,975, including thinnings and windfalls. Worminess in apples among varieties that were represented in each group, namely Yellow Newtown, Skinner Pippin, Hoover, and Ben Davis, from the unsprayed, wholly illuminated trees (eight) was 49.7 per cent, while the unsprayed check plot (not artificially illuminated) of eight trees showed 71.3 per cent worminess. The nonilluminated sprayed trees (fifteen) showed 7.4 per cent worminess. In spite of the fact that a higher artificial-light intensity had been used during the second season's work, the relative reduction in worminess for the entire test plot under illuminated and nonilluminated conditions was not greatly changed, i.e., the first season it showed a reduction of 31 per cent (illuminated 14.5 per cent and nonilluminated 21.0 per cent) while the second season showed a reduction of 30 per cent (illuminated 49.7 per cent and nonilluminated 71.3 per cent).

2. Using a single variety, namely Skinner Pippin, the conclusion is reached on the evidence at hand and under the conditions of this test that the percentage of worminess of apples in the more highly illuminated area is *much* less than the average for the several varieties combined, i.e., there is an apparent reduction of more than 50 per cent (illuminated 44.1 per cent; nonilluminated 89.1 per cent).

3. Since the codling moth deposits eggs rather freely within a light intensity range of from 0.3 to 90.0 foot-candles (station No. 2) with maximum activity between 25 and 52 foot-candles, and in view of the fact that an artificial-light intensity was maintained (station No. 6) ranging from 11 to 112 foot-candles at best, it becomes obvious that the

intensity of artificial light was not sufficiently high to wholly prevent codling moths from entering this area and depositing eggs. There was nevertheless a substantial decrease in moth attack as noted above.

4. It is evident that the effectiveness of artificial-light intensity in deterring moths remains fairly constant over a wide range of intensity, i.e.,  $30 \times 10^{-5}$  to  $1,560 \times 10^{-5}$ , when measured by values of  $1/D^2$ , as shown by the curve of worminess, and that it is not until the function of  $1/D^2$  falls below  $30 \times 10^{-5}$  that worminess increases abruptly.

### ACKNOWLEDGMENTS

As in the first test, so in this, the second, the author is particularly indebted to Mr. A. Halberg, owner of the orchard, for permission to use his trees for this investigation, and for the many courtesies which he extended throughout these tests. The writer is also greatly indebted to Mr. A. D. Borden, Assistant Entomologist, for his generous assistance in the field and to Dr. W. M. Hoskins for helpful criticism and advice in the study of the accumulated data. Particular mention should be made of the able assistance rendered by Mr. J. K. Ellsworth, Technical Assistant, who was stationed at the orchard throughout the period of the test and who also prepared the tables and drawings under the supervision of the author. Professor Ben D. Moses, Division of Agricultural Engineering and Director Secretary of the California Committee on the Relation of Electricity to Agriculture, has given most cordial support at all times.

The author is particularly indebted to the following members of the faculty who have read the manuscript and have contributed many helpful criticisms: Dr. H. B. Walker, Professor of Agricultural Engineering, Dr. S. C. Brooks, Professor of Zoology, and Dr. S. F. Cook, Associate Professor of Physiology.

# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

JANUARY, 1933

No. 8

## FIELD OBSERVATIONS ON THE BEET LEAFHOPPER, EUTETTIX TENELLUS, IN CALIFORNIA<sup>1</sup>

HENRY H. P. SEVERIN<sup>2</sup>

(Contribution from the Division of Entomology and Parasitology, California Agricultural Experiment Station, cooperating with the United States Department of Agriculture, Bureau of Entomology.)

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## INTRODUCTION

During 1918-1932 field investigations were carried on to determine where the beet leafhopper spends the winter and to locate the natural breeding areas in this state. Trips were taken to Death Valley, Mojave Desert, Imperial Valley, and the Tulare Lake and Bakersfield sections of the San Joaquin Valley—areas from which Ball<sup>(2)</sup> believed the beet leafhopper migrated into sugar-beet fields—and also to the middle and northern sections of the San Joaquin Valley, to the Sacramento Valley, Santa Clara, and Salinas valleys, and to all other important sugar-beet districts in California. After a general survey of this enormous territory, it soon became evident that it would require many years of field work to map the natural breeding areas of this insect, and hence the work was limited to the San Joaquin, Sacramento, Santa Clara, and Salinas valleys and to several small valleys. No foothill investigation has been carried on in Death Valley, Antelope Valley, Mojave Desert, and Imperial Valley. Such results as were obtained in these districts have been published in previous papers. <sup>(28, 30, 40)</sup>

The host plants of the beet leafhopper on the uncultivated plains and foothills, in the cultivated areas, and the original native host plants, the spring and summer dispersals and migrations, natural barriers, causes of fluctuation in population, and natural enemies were also observed during the investigations.

## DISTRIBUTION OF BEET LEAFHOPPER

The beet leafhopper is a native species and has been taken in western North America from Canada into Mexico. Davis<sup>(11)</sup> found the northern limit of the leafhopper was Cache Creek, British Columbia, 140 miles north of the international boundary. Carter<sup>(10)</sup> used the climograph as a means of comparing climates with respect to precipitation and temperatures in determining the probable limits of the range of the beet leafhopper; these studies were supplemented with surveys of the distribution of the insect. He considers that the presence of the leafhopper in British Columbia is due to a northward migration. Carter<sup>(9)</sup> published the results of the Canadian survey conducted by H. L. Seamans and states that the leafhopper was not found in the province of Alberta, although the results of the survey were not conclusive. Davis<sup>(11)</sup> found that the leafhopper was generally distributed in western Washington and Oregon. The area west of the Cascade Mountains in Oregon and

Washington represents the high-rainfall belt of the Pacific Northwest and is a limiting factor of the breeding range of the pest, although migrations into these areas occur.<sup>(10)</sup> Henderson<sup>(7)</sup> found that the beet leafhopper occurred in Lower California and western Mexico as far south as Guasave, Sinola. The insect was not found on the high central plateau, which extends from the southern portion of Durango to Mexico City.

### BREEDING AREAS

If nymphs of the spring generation are found year after year in a region, then that region can be considered as a permanent natural breeding area; on the other hand, when these nymphs are found only during a favorable year, that locality should be considered as a temporary breeding district. This leafhopper migrates long distances, and when insects migrate from their natural breeding grounds they fail to establish themselves in their new environment unless they meet conditions similar to their original habitat. In California the presence of pale-green adults of the spring generation, the pale-yellow forms of the summer generation, or the dark overwintering adults of the autumn generation, is no indication of a natural breeding area of the beet leafhopper. To map the natural breeding areas and migratory regions in some of the valleys in California required many years of field investigations.

*In San Joaquin Valley.*—The Great Interior Valley of California occupies the central part of the state. The valley is almost 400 miles long and from 20 to 50 miles wide and extends in a general northwest-southeast direction. It is bounded on its eastern side by the lower foothills of the Sierra Nevada Mountains and on its western side by the Inner Coast Range. The valley is enclosed around its northern end by the Klamath Mountains and around its southern end by the Tehachapi Mountains. The northern third of the Great Basin forms the Sacramento Valley and the remainder, the San Joaquin Valley. The San Joaquin and Sacramento valleys are not separated by a mountain barrier. The Marysville Buttes are located near the middle part of the Sacramento Valley, a few miles northwest of Marysville.

The northern limit of the breeding range of the beet leafhopper in the San Joaquin Valley was found to be in a canyon in the Inner Coast Range situated about 4 miles southwest of Pittsburg (fig. 1). The natural breeding area includes the canyons of the Inner Coast Range in the northern San Joaquin, the plains and foothills of the middle and southern San Joaquin, and the foothills of the Tehachapi Mountains.

The plains and foothills of most of Kern County are natural breeding grounds except the Sierra Nevada foothills near the northern end of the county. The northern limit of the breeding range on the Sierra Nevada foothills was found to be about 10 miles north of Porterville near Lindsay in Round Valley (fig. 1). The natural breeding area extends into the mountain passes of the Inner Coast Range for a distance of 12 miles in Little Panoche, Big Panoche, and Cholame passes.

*In Panoche Valley.*—A natural breeding area occurs between the Coast Ranges on the western foothills of the Panoche hills bounding Panoche Valley. When the hills are covered with shrubs and trees, as is

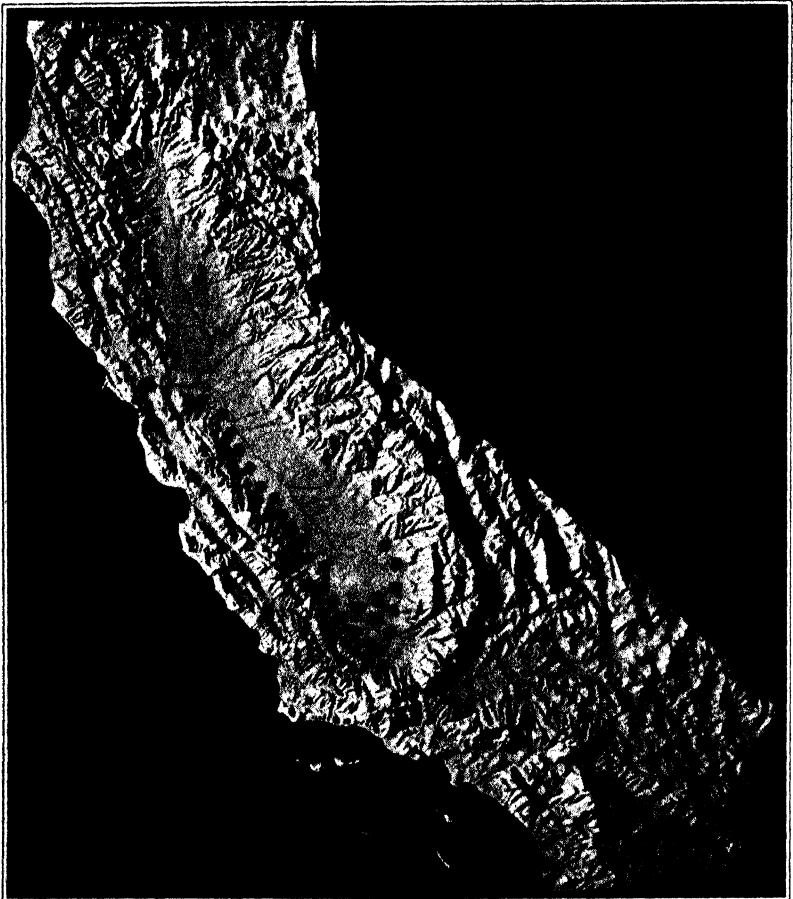


Fig. 1. Relief map of California showing natural breeding areas of beet leafhopper on plains and foothills in the San Joaquin Valley indicated by black dots. The northern third of the Great Basin represents the Sacramento Valley, which is a migratory area of the beet leafhopper.

the case on the Outer Coast Range, beet leafhoppers are rarely captured on red-stem filaree (*Erodium cicutarium*) (fig. 2), the most important breeding plant on the plains and foothills in California.

*In Salinas Valley.*—The Salinas Valley is the largest of the many valleys inclosed within the Coast Ranges in California. From Monterey Bay it extends in a southeasterly direction, in a line parallel with the

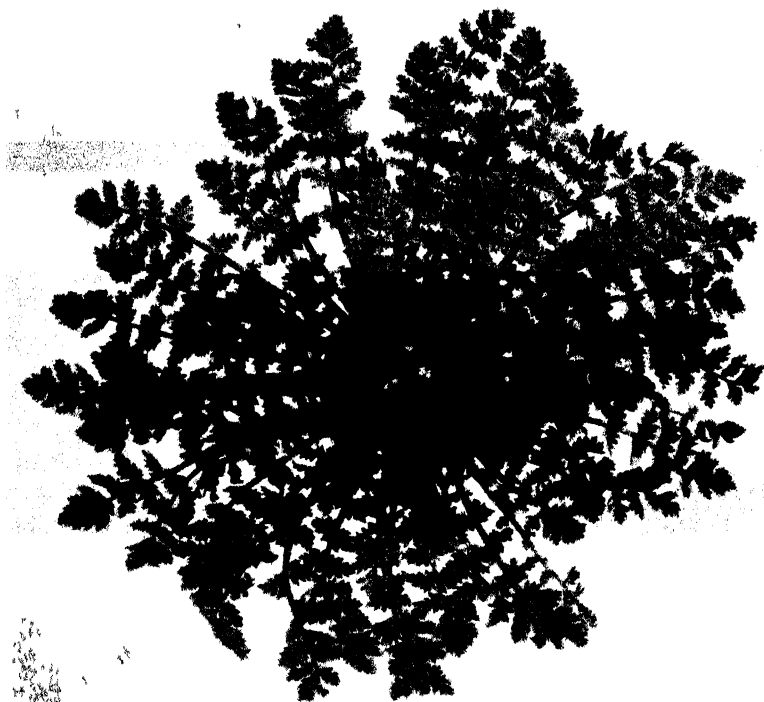


Fig. 2. Rosette form of red-stem filaree (*Erodium cicutarium*), which is the most important food and breeding plant on the plains and foothills, of the beet leafhopper in California.

coast, to its head, a few miles southeast of Santa Margarita—a distance of about 100 miles. Its average width is from 7 to 9 miles. Upon the northwest the valley is bounded by Monterey Bay; upon its sides by the Sierra Santa Lucia and Sierra Salinas ranges, with their outlying spurs upon the west; and by the Gabilan Mountains and Inner Coast Range upon the east, the latter separating the Salinas Valley from the San Joaquin Valley of the interior of the state.

The northern limit of the foothill breeding range on the Gabilan Mountains in the Salinas Valley is near the boundary of the fog belt south of Soledad, and the southern limit is in the vicinity of San Miguel,

the most favorable foothill breeding area extending from Greenfield to Bradley. During the autumn adults have been taken on the foothills of the Sierra Santa Lucia Mountains bounding the coastal side of the Salinas Valley and nymphs have been taken on the foothills about 5 miles southwest of King City, but this mountain range is only a minor natural breeding area.

*In Santa Ana Valley.*—Nymphs and adults were taken in the spring of 1926 on red-stem filaree growing on the southern exposures of the barren foothills in the Santa Ana Valley, south of Hollister. The eastern foothills were forested and no leafhoppers were taken.

*In Panoche and Pacheco Passes.*—During the spring of 1925 and 1926 a high population of nymphs and adults was found on the foothills in the vicinity of Paicines and in the western entrance of Panoche Pass, but no nymphs were taken in Pacheco Pass.

Suttie<sup>3</sup> found on November 24, 1931, from 10 to 15 overwintering adults to 100 sweeps of the insect net on perennials and red-stem filaree near Paicines and south toward the entrance of Panoche Pass. He also found on November 8, 1930, as many as 20 to 30 overwintering adults to 100 sweeps of the insect net on perennials growing along the San Benito River near Hollister.

A natural breeding area of the beet leafhopper extends from the Santa Ana Valley to Panoche Pass, becoming less favorable toward Pacheco Pass. In all probability the origin of the leafhopper in the Hollister beet district was from this natural breeding area and in some years through migrations from the San Joaquin Valley.

*In Santa Clara Valley.*—Investigations conducted during the autumn, winter, and spring of 1925–26 demonstrated that the beet leafhopper breeds on short red-stem filaree growing in rocky localities on the foothills extending out into the valley from two Coast Ranges in the southern part of the Santa Clara Valley. An occasional nymph and adult of the spring generation were taken on red-stem filaree in rocky localities on the foothills east of Coyote. This is a minor and temporary natural breeding area.

Suttie collected on January 11, 1927, an occasional overwintering adult on red-stem filaree in rocky localities on the foothills near Lick and Coyote.

*In Sierra Nevada Valleys.*—Natural breeding areas of the beet leafhopper occur in valleys located in the Sierra Nevada. Nymphs and adults were found on red-stem filaree growing sparingly on the rocky

<sup>3</sup> W. Suttie, of the Spreckels Sugar Company, in a personal interview with the author.

hillsides surrounding the southwestern portion of Honey Lake Valley (altitude 4,000 feet). Overwintering adults were taken in Sierra Valley (altitude 5,000 feet) during 1925, and adults of the spring generation were found in this valley by Hartung during 1919 and Schwing during 1924, as reported in a previous paper.<sup>(32)</sup> The leafhopper and curly top of sugar beets were also reported in the same paper as occurring in Indian Valley (altitude 3,600 feet) and American Valley (altitude 3,400 feet).

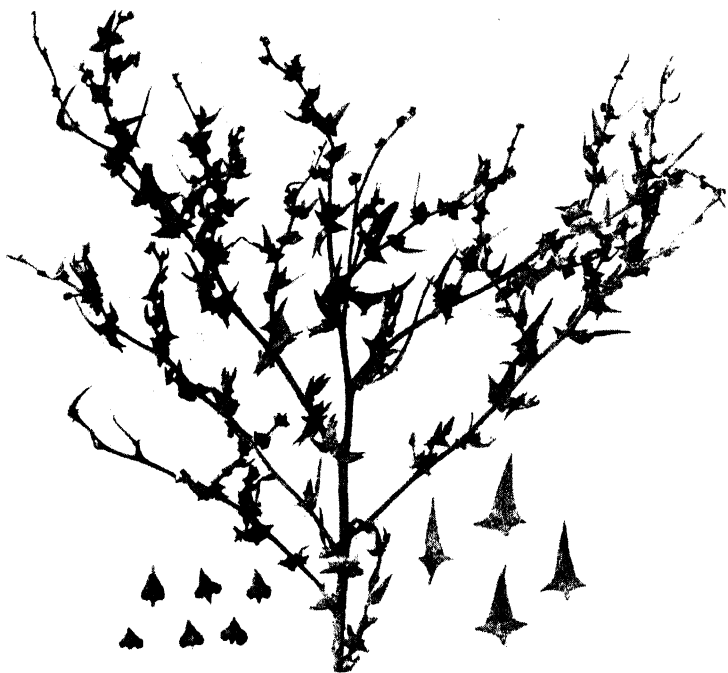


Fig. 3. Branch of arrowscale (*Atriplex phyllostegia*), showing leaves and fruiting bracts, also characteristic leaves and fruiting bracts removed from plant.

## HOST PLANTS

### ON UNCULTIVATED PLAINS AND FOOTHILLS

*Food Plants.*—There existed on the plains and foothills of California an abundance of grasses, clovers, and wild flowers, until man disturbed the native conditions. As early as 1773, the Spaniards disturbed the native conditions by introducing sheep which carried in their wool seeds of plants from the Mediterranean Basin. The ensuing competition between the native and introduced plants greatly diminished most of the native species.

Red-stem filaree, which occurs on the barren hillsides and dry plains has been considered an introduced species, but there is some difference of opinion among botanists. If this plant was introduced, then a special adaptation of the leafhopper to it has occurred.

Cattle and sheep have overgrazed the preferred introduced forage plants, so that these were not permitted to produce seeds abundantly. It is these overgrazed foothills sparsely covered with red-stem filaree in the semiarid regions that are the most favorable habitat of this insect.



Fig. 4. Twigs of fleshscale or Australian saltbush (*Atriplex semibaccata*), showing leaves and fruiting bracts, also different-shaped leaves and fruiting bracts removed from plant. This perennial saltbush was introduced into California about thirty years ago from Australia as a forage plant, and is one of the most important food and breeding plants of the beet leafhopper in the Imperial Valley. It is also a favorable host plant of the leafhopper in the San Joaquin and Salinas valleys, but is not abundant in the Sacramento Valley. It is well established in the southern part of the state and is common in the fog belt.

The disturbance of the native conditions on the plains and foothills and in the cultivated areas increased the most favorable food and breeding plants of the leafhopper and hence has increased the opportunities for an enormous multiplication of the pest when climatic conditions are favorable.

The relative numbers of beet leafhoppers captured on plants growing on the plains and foothills of the Coast Range bounding the San Joaquin Valley were determined during 1918. The insects were taken on twenty species of plants, five of which belong to the saltbush family. The reader is referred to a previous paper<sup>(28)</sup> for a list of food plants.

**Breeding Plants.**—The beet leafhopper has been bred from five different species of annual plants growing on the foothills of the San Joaquin Valley: red-stem filaree (*Erodium cicutarium*) and white-stem filaree or musk filaree (*E. moschatum*) (Geraniaceae), *Hollisteria lanata* (Polygonaceae), *Malvastrum exile* (Malvaceae), and common peppergrass (*Lepidium nitidum*) (Cruciferae). Red-stem filaree is the most impor-

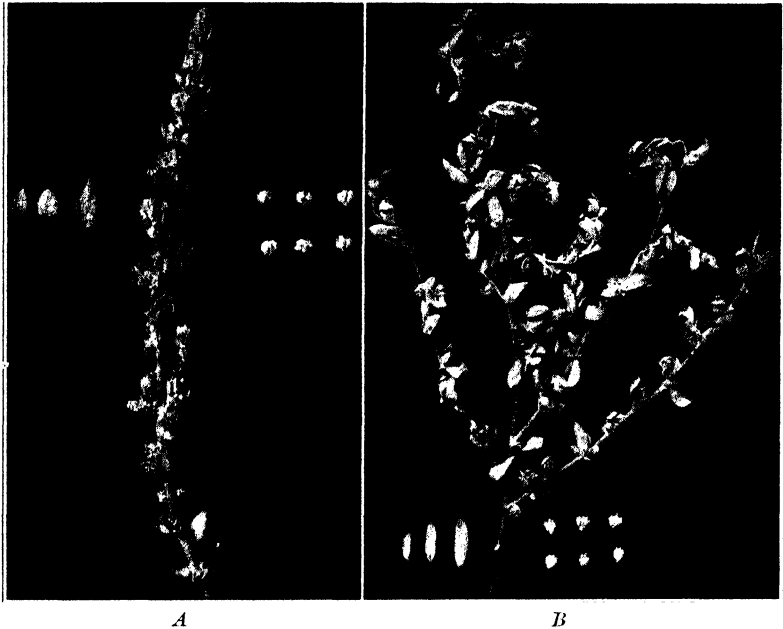


Fig. 5. *A*, Branch of crownscale (*Atriplex coronata*), showing leaves and fruiting bracts, also leaves and fruiting bracts removed from plants. *B*, Branch of ballscale (*Atriplex fruticulosa*), showing leaves and fruiting bracts, also leaves and fruiting bracts removed from plant. During dry autumns before the pasture vegetation has germinated, beet leafhoppers are commonly taken on this plant on the plains of the San Joaquin Valley.

tant host plant upon which the overwintering adults feed and deposit their eggs, and upon which the spring generation develops.

The nymphs hatched from eggs deposited in the leaves or stems of the following perennial Chenopodiaceae growing on the plains of the San Joaquin Valley: ballscale (*Atriplex fruticulosa*), spinescale (*A. spinifera*), and Australian saltbush or fleshscale (*A. semibaccata*).

#### IN CULTIVATED AREAS

**Food Plants.**—The relative numbers of beet leafhoppers captured on plants growing in the cultivated areas of the San Joaquin Valley were determined in 1918. The insects were taken on thirty species of



plants,<sup>(28)</sup> eighteen of which belong to the Chenopodiaceae. After the invasion of the cultivated areas by the pest, the leafhoppers were more abundant on plants of the family Chenopodiaceae, to which the sugar beet belongs.

During the spring the beet leafhopper was found in enormous numbers on short-lived annual saltbushes, such as arrowscale (*Atriplex*



Fig. 6. *A*, Branch of silverscale or fogweed (*Atriplex argentea expansa*), showing leaves and fruiting bracts. *B*, Branch of redscale or red orache (*Atriplex rosea*), showing leaves and fruiting bracts, also fruiting bracts removed from plant.

*phyllostegia*) (fig. 3; plate 1, *B*); heartscale (*A. cordulata*) (plate 1, *F*; plate 3, *C*); crownscale (*A. coronata*) (fig. 5 *A*; plate 1, *E*); brittlescale (*A. parishii*) (plate 1, *I*; plate 3, *A*); but during the summer when these saltbushes become dry except in irrigated fields, the leafhoppers assemble on other favorable host plants. It was frequently noticed that when the stems of other species of plants became woody the insects left, but this was not the case with fogweed or silverscale (*A. argentea expansa*) (fig. 6*A*; plate 1, *A*); red orache or redscale (*A. rosea*) (fig. 6*B*; plate 1, *C*); bractscale (*A. bracteosa*) (plate 1, *D*; plate 3, *D*); and Russian



Fig. 7. Vacant field covered with bractscale (*Atriplex bracteosa*).



Fig. 8. Bractscale (*Atriplex bracteosa*) growing along a fence, showing height of plant, which may vary from 1 to 15 feet, and with stems commonly spreading to form dense tangled mats from 1 to 10 feet across, from which arise slender erect or ascending twigs.

This annual saltbush forms dense communities in the San Joaquin Valley, and owing to its size and abundance, is one of the most important food and breeding plants of the beet leafhopper in the cultivated areas of California. The leafhoppers are commonly found on this weed from the time that the spring dispersal from the plains and foothills begins until the return flights occur.

thistle (*Salsola kali tenuifolia*). The leafhoppers remained on these plants until the leaves became dry.

Wherever man has disturbed the native conditions in the San Joaquin Valley, vast areas of annual saltbushes occur. Saltbushes grow abundantly along fences (fig. 8), roadsides, highways (fig. 9), and railroad tracks (fig. 10), on vacant fields (fig. 7), and, after the grain is harvested, on stubble fields, and commonly around hay and straw stacks. Dense masses surround alkali sinks (fig. 11), although the black alkali is often too strong for their development. Irrigation and drainage



Fig. 9. Bractscale (*Atriplex bracteosa*), growing along highway.

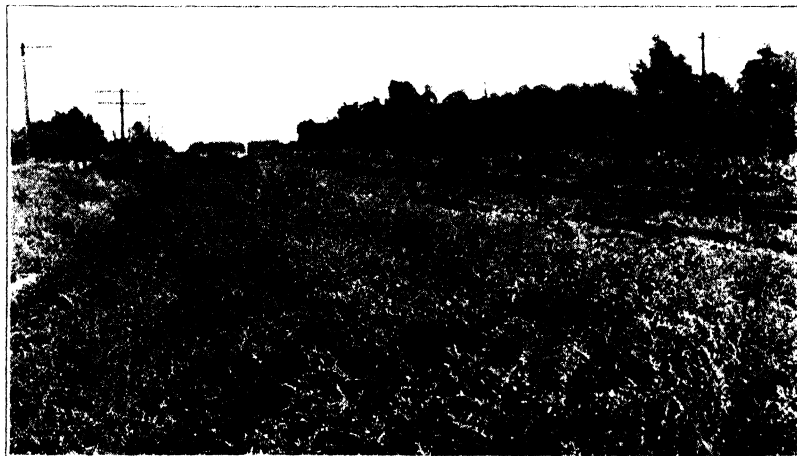


Fig. 10. Silverscale or fogweed (*Atriplex argentea expansa*), growing along railroad tracks.

canals are favorable locations for the development of this alkali vegetation (fig. 13).

The stimulus for the development of enormous areas of annual saltbushes in the San Joaquin Valley has probably been the increase of alkali lands by man's activities. According to Kelly<sup>(23)</sup> "several hundred thousand acres in the San Joaquin Valley, which were comparatively free from alkali previous to the advent of irrigation, have already been seriously injured, or abandoned." He states that alkali finds its way into good lands by the use of saline irrigation water, and by the rise of the ground-water level through seepage and overirrigation.



Fig. 11. Alkali sink surrounded by dense growths of redscale or red orache (*Atriplex rosea*).

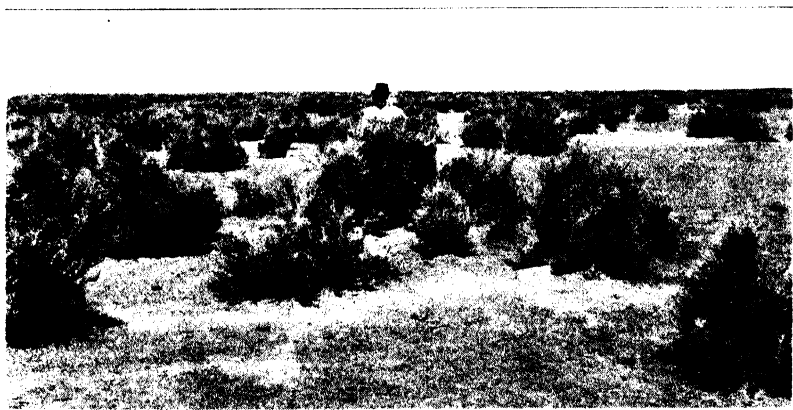


Fig. 12. Plains of the western San Joaquin Valley covered with spinescale (*Atriplex spinifera*), a perennial saltbush, which serves as a food plant of the beet leafhopper during dry autumns and winters before the pasture vegetation has germinated.

*Breeding Plants.*—The most important factor in the enormous increase of the beet leafhopper in the San Joaquin Valley is the abundance of the breeding plants in the cultivated areas. The plants upon which enormous numbers of nymphs and adults were taken in the field are representatives of the Chenopodiaceae. The leafhopper was bred from eggs deposited under natural conditions in thirty-eight species of weeds growing in the cultivated regions. The weeds upon which adults were collected were removed with the root system from vacant fields, stubble



Fig. 13. Bractscale (*Atriplex bracteosa*), growing along an irrigation canal.

fields, beet fields, truck-crop fields, along roadsides, railroad tracks, rivers, and irrigation and drainage canals. Table 1 lists the plants in which the beet leafhopper deposited eggs in the cultivated districts and shows which are native to this country and which introduced from other countries.

Later investigations have shown that the nymphs are not able to acquire the winged stage by feeding on some of the weeds in which eggs are deposited. The breeding experiments indicate that the most favorable host plants in the cultivated areas are plants of the Chenopodiaceae, to which the sugar beet belongs.

The host plants of the beet leafhopper among economic plants was reported in previous papers. <sup>(34, 37)</sup>

TABLE 1

LIST OF WEEDS IN WHICH BEET LEAFHOPPERS DEPOSITED EGGS IN CULTIVATED AREAS UNDER NATURAL CONDITIONS IN CALIFORNIA\*

Common name	Scientific name	Family	Origin	Valley in which breeding plants were obtained
Annual saltbushes				
Arrowscale	<i>Atriplex phyllostegia</i> Wats.	Chenopodiaceae	Native	San Joaquin
Bractscale	<i>Atriplex bracteosa</i> Wats.	Chenopodiaceae	Native	San Joaquin, Sacramento, Salinas
Brittlescale	<i>Atriplex parishi</i> Wats.	Chenopodiaceae	Native	San Joaquin, Sacramento
Crownscale	<i>Atriplex coronata</i> Wats.	Chenopodiaceae	Native	San Joaquin, Sacramento
Fogweed (silverscale)	<i>Atriplex argentea expansa</i> (Wats.)	Chenopodiaceae	Native	San Joaquin, Sacramento, Salinas
Heartscale	<i>Atriplex cordulata</i> Jepson	Chenopodiaceae	Native	San Joaquin, Sacramento
Spear orache (spearscale)	<i>Atriplex patula</i> L.	Chenopodiaceae	Native	Sacramento
Wheelscale	<i>Atriplex tularensis</i> Coville	Chenopodiaceae	Native	San Joaquin
Red orache (redscale)	<i>Atriplex elegans</i> (Moq.) Dietr. <i>Atriplex rosea</i> L.	Chenopodiaceae Chenopodiaceae	Native Introduced	Imperial San Joaquin, Sacramento, Salinas
Perennial saltbushes				
Australian saltbush (fleshyscale)	<i>Atriplex semibaccata</i> R. Br.	Chenopodiaceae	Introduced	San Joaquin, Sacramento, Salinas, Imperial
Pigweeds				
.....	<i>Chenopodium leptophyllum</i> Wats.	Chenopodiaceae	Native	San Joaquin
Lamb's quarters (white pigweed)	<i>Chenopodium album</i> L.	Chenopodiaceae	Introduced	San Joaquin, Sacramento, Salinas
Sowbane (nettle- leaf goosefoot)	<i>Chenopodium murale</i> L.	Chenopodiaceae	Introduced	San Joaquin, Sacramento
Mexican tea	<i>Chenopodium ambrosioides</i> L.	Chenopodiaceae	Introduced	San Joaquin, Sacramento
Other weeds				
.....	<i>Nitrophila occidentalis</i> (Moq.) Wats.	Chenopodiaceae	Native	San Joaquin, Sacramento
Russian thistle (Continued)	<i>Salsola kali</i> L. var. <i>tenuifolia</i> G. F. W. Mey.	Chenopodiaceae	Introduced	San Joaquin, Sacramento, Salinas

\* The saltbushes were determined by H. M. Hall, Carnegie Institution of Washington, and all other weeds were classified by various systematists of the Division of Botany, University of California.

TABLE 1—(Concluded)

Common name	Scientific name	Family	Origin	Valley in which breeding plants were obtained
Other Weeds—(Continued)				
.....	<i>Suaeda depressa</i> (Pursh) var. <i>erecta</i> Wats.	Chenopodiaceae	Native	San Joaquin
Curly dock	<i>Rumex crispus</i> L.	Polygonaceae	Introduced	San Joaquin, Sacramento, Salinas
Wire grass (yard grass)	<i>Polygonum aviculare</i> L.	Polygonaceae	Introduced	San Joaquin
Rough pigweed	<i>Amaranthus retroflexus</i> L.	Amaranthaceae	Introduced	San Joaquin, Sacramento, Salinas
Tumbleweed	<i>Amaranthus graecizans</i> L.	Amaranthaceae	Introduced	San Joaquin, Salinas
.....	<i>Amaranthus deflexus</i> L.	Amaranthaceae	Introduced	San Joaquin, Salinas
Indian chickweed	<i>Mollugo verticillata</i> L.	Aizoaceae	Native	San Joaquin
Lowland purslane	<i>Securium sessile</i> Pers.	Aizoaceae	Native	San Joaquin, Imperial
Red maids	<i>Calandrina caulescens</i> H. B. K. var. <i>menziesii</i> Gray	Portulacaceae	Native	San Joaquin
Common purslane	<i>Portulaca oleracea</i> L.	Portulacaceae	Introduced	San Joaquin
Charlock	<i>Brassica arvensis</i> (L.) B. S. P.	Cruciferae	Introduced	San Joaquin, Sacramento
Wild radish	<i>Raphanus sativus</i> L.	Cruciferae	Introduced	Salinas
Jackass clover	<i>Wislizenia refracta</i> Engelm.	Cruciferae	Native	San Joaquin
Spanish clover	<i>Lotus americanus</i> (Nutt.) Bisch.	Leguminosae	Introduced	San Joaquin
Dwarf mallow	<i>Malva rotundifolia</i> L.	Malvaceae	Introduced	Salinas
Cheese weed	<i>Malva parviflora</i> L.	Malvaceae	Introduced	San Joaquin
Alkali mallow	<i>Sida hederacea</i> (Dougl.) Torr.	Malvaceae	Native	San Joaquin
Orchard morning-glory	<i>Convolvulus arvensis</i> L.	Convolvulaceae	Introduced	Sacramento
Chinese pusley	<i>Heliotropium curassavicum</i> L.	Boraginaceae	Native	San Joaquin, Salinas
Horehound	<i>Marrubium vulgare</i> L.	Labiatae	Introduced	Salinas
Tolguacha (jimson weed)	<i>Datura meteloides</i> D. C.	Solanaceae	Native	San Joaquin, Salinas
.....	<i>Solanum douglasii</i> Dunal.	Solanaceae	Native	Salinas
Horseweed	<i>Erigeron canadensis</i> L.	Compositae	Native	San Joaquin
Cotton-batting plant	<i>Gnaphalium chilense</i> Spreng.	Compositae	Native	Sacramento
Common sunflower	<i>Helianthus annuus</i> L.	Compositae	Native	San Joaquin
Common spike-weed	<i>Centromadia pungens</i> (T. & G.) Greene	Compositae	Native	San Joaquin
Spiny clotbur	<i>Xanthium spinosum</i> L.	Compositae	Introduced	San Joaquin, Salinas
Mayweed	<i>Anthemis cotula</i> L.	Compositae	Introduced	San Joaquin

## ORIGINAL NATIVE HOST PLANTS

The distribution of insects is often limited by the geographical range of native food and breeding plants. The most important host plants of the beet leafhopper are representatives of the Chenopodiaceae (saltbush family) and Cruciferae (mustard family). H. M. Hall has prepared a brief statement concerning the geographical distribution of the species of saltbushes as follows: "There are numerous species of *Atriplex* (saltbushes) throughout western North America as far south as tropical Mexico, the number of species as well as individuals diminishing toward the south."

*Perennials*.—As shown in table 2, *Atriplex nuttalli* and *A. nuttalli falcata* are the only perennial saltbushes which serve as breeding plants of the beet leafhopper in states other than California; all others are food plants.

The beet leafhopper occurs abundantly on certain perennial saltbushes in the San Joaquin Valley during dry autumns. There are on the plains of the western San Joaquin Valley vast areas of shrubby perennial saltbushes (fig. 12). A comparison of the natural breeding grounds of the leafhopper with the distribution of the perennial saltbushes in valleys where an intensive study of both has been made follows.

Cattle spinach or allscale (*Atriplex polycarpa*) (fig. 14A; plate 2, B) is one of the most favorable shrubby perennial saltbushes as a food plant of the leafhopper. According to W. C. Cook it was found in Los Banos Creek, 10 miles southwest of Los Banos in the middle San Joaquin Valley, and extends as far south as the Tehachapi Mountains. This saltbush is green during dry autumns. Lower populations of leafhoppers are obtained when *A. polycarpa* was swept during the afternoon than after sunset. In one test two persons swept these shrubs with an insect net for one hour from 2:40 to 3:40 P.M. and 24 specimens were taken. The same saltbushes were swept for half an hour from 5:15 to 5:45 P.M. and 126 specimens were taken. In all probability the leafhoppers remain within the shrubs during the daytime, and come to the outer branches and foliage at sunset.

Spinescale (*Atriplex spinifera*) (fig. 12; plate 2, A) which is sometimes mixed with *A. polycarpa*, is often too dry for large populations of the leafhopper in October, but during November it develops fresh leaves and is a favorable food plant. *A. spinifera* occurs as far north as Volta in the San Joaquin Valley and has been recorded as far south as Buena Vista hills in Kern County. During the 1919 outbreak of the beet leaf-



TABLE 2

PLANTS AMONG THE CHENOPODIACEAE AND CRUCIFERAE USED AS FOOD OR BREEDING  
PLANTS BY THE BEET LEAFHOPPER

State	Authority	Common name	Scientific name	Origin
Chenopodiaceae, perennial saltbushes				
Idaho	Haegele <sup>(15)</sup>	Shadscale	<i>Atriplex confertifolia</i> (Torr. & Frem.) Wats.	Native
		Moundscale	<i>Atriplex muttalli</i> Wats.	Native
		Wingscale	<i>Atriplex pabularis</i> Nels.	Native
		Shadscale	<i>Atriplex canescens</i> (Pursh) Nutt	Native
Utah	Knowlton <sup>(24,25)</sup>	Quailbrush, lenscale	<i>Atriplex lentiformis</i> (Torr.) Wats.	Native
		Moundscale	<i>Atriplex nuttalli</i> Wats.*	Native
		Moundscale	<i>Atriplex nuttalli falcata</i> (Jones)*	Native
		Cattle spinach, allscale	<i>Atriplex polycarpa</i> (Torr.) Wats.	Native
		Wingscale	<i>Atriplex canescens</i> (Pursh) Nutt.	Native
		Ballscale	<i>Atriplex fruticulosa</i> Jepson*	Native
California	Severin <sup>(28,29,30)</sup>	Quailbrush, lenscale	<i>Atriplex lentiformis</i> (Torr.) Wats.	Native
		Cattle spinach, allscale	<i>Atriplex polycarpa</i> (Torr.) Wats.	Native
		Australian saltbush	<i>Atriplex semibaccata</i> Brown*	Introduced
		Spinescale	<i>Atriplex spinifera</i> McBr.*	Native
Chenopodiaceae, annual saltbushes				
Washington	Ball <sup>(2)</sup>	.....	<i>Atriplex</i> sp. (?)	Native
Oregon	Ball <sup>(2)</sup>	.....	<i>Atriplex</i> sp. (?)	Native
Idaho	Haegele <sup>(15)</sup> Carter <sup>(10)</sup>	Red orache, redscale	<i>Atriplex rosea</i> L.*	Introduced
		Red orache, redscale	<i>Atriplex rosea</i> L.*	Introduced
		Silverscale	<i>Atriplex argentea</i> Nutt.*	Native
		Gardenscale, garden orache	<i>Atriplex hortensis</i> L.	Introduced(?)
Utah	Knowlton <sup>(24,25)</sup>	Spearscale, spear orache	<i>Atriplex patula hastata</i> (L.)	Native
		Ribscale	<i>Atriplex powelli</i> Wats.	Native
		Red orache, redscale	<i>Atriplex rosea</i> L.*	Introduced
		Wedgescale	<i>Atriplex truncata</i> (Torr.) Gray*	Native
Isla Raza Santa Inez Island	Van Duzee <sup>(45)</sup>	.....	<i>Atriplex</i> sp. (?)	.....
		.....	.....	.....
		.....	<i>Atriplex</i> sp. (?)	.....
Cruciferae				
Idaho	Haegele <sup>(16)</sup>	Wormseed mustard	<i>Erysium cheiranthoides</i> L.	Introduced
		Tumbling mustard	<i>Norta altissima</i> (L.) Britt.*	Introduced
		Green tansy mustard	<i>Sophia filipes</i> (Gray) Heller*	Native
		Penny cress, fan-weed	<i>Thlaspi arvensis</i> L.	Introduced
(Continued)				

\* Breeding plants; all others are food plants.

TABLE 2—(Concluded)

State	Authority	Common name	Scientific name	Origin
Cruciferae—(Concluded)				
Idaho	Carter <sup>(10)</sup>	Flixweed, herb-sophia	<i>Sophia sophia</i> (L.) Britt.*	Introduced
		.....	<i>Sophia filipes</i> (Gray) Heller*	Native
		Jim Hill mustard	<i>Norta altissima</i> (L.) Britt.*	Introduced
		Black mustard	<i>Brassica nigra</i> (L.) Koch	Introduced
		Shepherd's purse	<i>Bursa bursa-pastoris</i> (L.) Britt.	Introduced
		False flax	<i>Camelina microcarpa</i> Andrezej	Introduced
		Blister cress	<i>Cheirinia repanda</i> (L.) Link*	Introduced
		Blister cress	<i>Cheirinia cheiranthoides</i> (L.) Link	Introduced
		Blister cress	<i>Cheirinia aspera</i> (Nutt.) Rydb.	Native
		Malcolmia	<i>Malcolmia afrienna</i> (L.) R. Br.*	Introduced
		Pepper grass	<i>Lepidium perfoliatum</i> L.	Introduced
		Pepper grass	<i>Lepidium pubicarpum</i> A. Nels.	Native
		Pepper grass	<i>Lepidium ramosum</i> A. Nels.	Native
		Pepper grass	<i>Lepidium densiflorum</i> Schrad.*	Native
		Tumble mustard	<i>Norta altissima</i> (L.) Britt.*	Introduced
Utah	Knowlton <sup>(24,25)</sup>	Whitlow grass	<i>Draba micrantha</i> Nutt.	Native
		Whitlow grass	<i>Draba nemorosa</i> L.	Native
		Whitlow grass	<i>Draba cuneifolia</i> Nutt.	Native
		Water cress	<i>Sisymbrium nasturtium-aquaticum</i> L.	Introduced
		Schoenocrambe	<i>Schoenocrambe linifolia</i> (Nutt.) Greene	Native
		Charlock	<i>Sinapsis arvensis</i> L.	Introduced
		Tansy mustard	<i>Sophia sonne</i> (Rob.) Greene	Native
		Tansy mustard	<i>Sophia filipes</i> (A. Gray) Heller*	Native
		Tansy mustard	<i>Sophia pinnata</i> (Walt.) Howell*	Native
		Tansy mustard	<i>Sophia hartwegiana</i> (Fourn.) Greene	Native
		Tansy mustard	<i>Sophia incisa</i> (Engelm.) Greene	Native
		Tansy mustard	<i>Sophia longipidicellata</i> (Fourn.) Howell	Native
		Tansy mustard	<i>Sophia procera</i> Greene	Native
		Tansy mustard, flixweed	<i>Sophia sophia</i> (L.) Britt.*	Introduced
			<i>Sophia parviflora</i> (Lam.) Standl.*	Introduced

\* Breeding plants; all others are food plants.

hopper, nymphs hatched from eggs deposited in the leaves of this perennial saltbush under natural conditions.

Quailbrush or lenscale (*Atriplex lentiformis*) (fig. 14 B; plate 2, C) is not as favorable a food plant as the previous two species. *A. lentiformis* is more tolerant to alkali and occurs on alkaline flats and river benches and according to Hall and Clements<sup>(16)</sup> is the next stage in the succession with *A. polycarpa* and *A. spinifera*. *A. lentiformis* occurs as far north as Firebaugh and south to the Tehachapi Mountains.

In the Sacramento Valley no shrubby perennial saltbushes were found. Ballscale (*Atriplex fruticulosa*) (fig. 5B; plate 2, E), a small

perennial, occurs in the Sacramento Valley from Glenn County south in the San Joaquin Valley. It occurs on both the plains and foothills in the San Joaquin Valley, and leafhoppers are commonly taken on it during dry autumns.

Australian saltbush or fleshscale (*Atriplex semibaccata*) (fig. 4; plate 2, *D*) is common along roadsides, irrigation canals, and fallow fields,



Fig. 14. *A*, Cattle spinach or allscale (*Atriplex polycarpa*), showing end of branch with leaves and fruiting bracts. *B*, Quailbrush or lenscale (*Atriplex lentiformis*), showing end of branch with leaves and fruiting bracts, also fruiting bracts removed from plant.

but is not abundant on the uncultivated plains. The leafhoppers are frequently abundant on it during dry autumns. In some years its foliage was destroyed by heavy frosts, but in a warm winter high percentage of males were taken on several acres of it near Wasco as follows: December 10, 1918, 75 per cent and February 16, 1919, 82 per cent. During the 1925 outbreak of the beet leafhopper large numbers of nymphs and adults were found during the summer on *A. semibaccata* growing on the hillsides of the Gabilan Mountains near Metz in the Salinas Valley. The leafhopper was abundant on it during the winter in the Imperial Valley.

The only native shrubby perennial saltbush in the Salinas Valley is *Atriplex lentiformis*, which is not a favorable food plant of the beet leafhopper. It is found in rare patches from Metz south in the valley.

In the natural breeding area of the Santa Ana Valley to the western entrance of Panoche Pass no native perennial saltbushes were found.

The limited distribution of *Atriplex lentiformis* in the Salinas Valley and the absence of native perennial saltbushes in the Santa Ana Valley indicates that the perennial saltbushes were probably not the original native host plants, but simply serve as food plants during dry autumns in California. It could be argued, however, that the beet leafhopper extended its range to the Santa Ana and Salinas valleys, through migrations from the San Joaquin Valley, after the red-stem filaree spread to the foothills.

During the autumn dispersal, leafhoppers are commonly taken on other shrubby perennials growing on the plains and foothills in the San Joaquin Valley, such as *Gutierrezia californica* and *Isocoma veneta*, which are 1 to 2 feet high. Creek senecio (*Senecio douglasii*), which grows to a height of 2 to 6 feet, is another food plant of the overwintering adults during dry autumns, but it is limited in its distribution. It does not form distinct communities and occurs on gravelly plains and dry stream beds in the foothills of the San Joaquin Valley and sandy floor of the Salinas River and its tributaries. In the Salinas Valley the leafhoppers are common on *Lepidospartum squamatum*, a rigid broom-like shrub from 3 to 6 feet high growing in the stream bed of the Salinas River and its tributaries. *L. squamatum* often forms dense stands in canyons and in some of the mountain passes in the San Joaquin Valley, and owing to its wide distribution is one of the most important food plants of the beet leafhopper during dry autumns. There are many other perennials on which the overwintering adults are taken during dry autumns in the San Joaquin and Salinas valleys, and when the insects are abundant they are taken on all green vegetation.

**Mortality on Perennials.**—A change in food plants from favorable annuals in the cultivated areas to perennials on the plains and foothills may result in a high mortality of the overwintering adults during the autumn dispersal. No experiments have been conducted up to the present time on the longevity of the overwintering adults on different species of perennials. The longevity of the last living male and female beet leafhopper was reported in previous papers<sup>(33, 34, 37)</sup> on the host range of curly top. It was frequently found that nymphs which hatched from eggs deposited in host plants would acquire the winged stage on food plants that were unfavorable to the adults.

The overwintering adults have well-developed fat bodies and the food requirements are probably not as important as the water requirements during the early autumn. Carter<sup>(8)</sup> has been able to sustain the life of the insect on tap water for long periods.

*Annuals.*—Surveys have been made of the host plants of the beet leafhopper in a number of states, and a discussion of the most important host plants in Canada and the United States follows.

Davis<sup>(11)</sup> collected the beet leafhopper on Russian thistle and mangels in British Columbia and western Washington, and on these same plants and beets in western Oregon.

In Idaho Haegele<sup>(15)</sup> found that the principal and most widespread host plants of the Chenopodiaceae were *Atriplex rosea*, Russian thistle (*Salsola pestifer*), and *Bassia hirsuta*. Tumbling mustard (*Norta altissima*) and green tansy mustard (*Sophia filipes*) of the Cruciferae were also important hosts of the beet leafhopper during the spring.

Carter<sup>(10)</sup> found a sequence of host plants of the beet leafhopper throughout the season in central-southern Idaho including three species of mustards (*Sophia sophia*, *Sophia filipes*, and *Norta altissima*), *Atriplex rosea*, and *Salsola pestifer*, together with occasional hosts such as *Solanum triflorum*. *Salsola pestifer* is the most important late summer and fall host.

Knowlton<sup>(25)</sup> considers that the most important spring host plants in northern Utah breeding grounds are the following species of Cruciferae: *Cherinia repanda*, *Norta altissima*, *Sophia sophia*, and *Lepidium perfoliatum*; and also *Erodium cicutarium*, a representative of the family Geraniaceae. The two most important summer host plants are *Salsola pestifer* and *Atriplex rosea*. The spring host plants listed above again become important fall hosts, carrying the beet leafhoppers until cold weather puts an end to activity.

The most important breeding plant among the annual saltbushes in states other than California is *Atriplex rosea*, an introduced species. It is only the native host plants that can be considered as the original host plants of the beet leafhopper. Among the important California annual saltbushes on which the summer and autumn generations of beet leafhopper develop are *Atriplex argentea expansa*, *A. bracteosa*, *A. patula*, and *A. rosea*. The latter becomes dry too early in the fall to maintain large populations of the autumn generation. *Atriplex phyllostegia*, *A. parishii*, *A. coronata*, *A. cordulata*, and *A. elegans* (plate 1, fig. *H*) are short-lived annual saltbushes which usually become dry in July and on which the summer generation of the beet leafhopper develops.

Among the Cruciferae, green tansy mustard (*Sophia filipes* or, according to Jepson<sup>(22)</sup> *Sisymbrium incisum* var. *filipes*) is a native species on which the beet leafhopper breeds abundantly in Idaho and Utah, but this species (or variety) does not occur in California. In Utah Knowlton<sup>(25)</sup> has captured nymphs on tansy mustard (*Sophia pinnata*), a native species, but it has not been shown to be a breeding plant of the beet leafhopper in California. Nymphs have been taken on the native pepper grass (*Lepidium densiflorum*) in Utah, but this species does not occur in California. In California nymphs were bred from eggs deposited in common pepper grass (*Lepidium nitidum*) collected in Little Panoche Pass. This species is common on the California plains, low hills, and in the valleys, and extends north to Washington. Further breeding experiments are necessary to determine whether the native mustards were the original host plants on which the spring generation developed.

Davis<sup>(11)</sup> failed to take the beet leafhopper on red-stem filaree in British Columbia, western Washington, and western Oregon, but his collections were made from August 10 to September 15, 1926, when this plant would probably not serve as a host plant of the insect. According to Haeghele,<sup>(15)</sup> the beet leafhopper was not found on red-stem filaree, which is comparatively rare in Idaho. In northern Utah Knowlton<sup>(25)</sup> found, as a rule, low populations of the beet leafhopper on red-stem filaree during the spring, but in a few instances high populations were encountered on this plant during the autumn.

It has been generally considered by botanists that red-stem filaree was introduced from the Mediterranean Basin by the Spaniards into Mexico and South America perhaps as early as the sixteenth century, and a little later into California. This plant belongs to the geranium family (Geraniaceae), which is by no means closely related to the salt-bush family (Chenopodiaceae). The possibility that the beet leafhopper was accidentally introduced in red-stem filaree from Europe is entirely out of consideration, for, if this plant was introduced into America from the Mediterranean Basin, it was almost certainly through the seeds carried in the wool of sheep.

Two botanists, however, express a different opinion as to the native home of red-stem filaree. According to Brewer and Watson,<sup>(6)</sup> *Erodium cicutarium* is "very common throughout the state, extending to British Columbia, New Mexico, and Mexico, also widely distributed in South America and the Eastern Continent. It has been generally considered as an introduced species, but is more decidedly and widely at home throughout the interior than any other introduced plant, and according

to much testimony it was as common throughout California early in the present century as now."

Hendry and Kelly<sup>(21)</sup> found in adobe bricks the seeds or portions of plants of 32 different weeds, 11 of which were recognized by botanists as introduced European species. In the construction of the adobe bricks weeds of all kinds were used as a binder, particularly those with fibrous stems, including filaree. Red-stem filaree (*Erodium cicutarium*) was found in various adobe structures of missions constructed between 1797 and 1834 in various localities of California. They state: "The mere finding of these plants does not prove that they were introduced by the missions, but when the same weed occurs repeatedly in widely separated localities, and when it is remembered that many of these weeds characteristically follow in the wake of cultivation, it would seem that the missions must have played an important part in their dissemination, if not in their actual introduction."

If red-stem filaree was introduced into California, then in all probability the original native host plants, on which the overwintering adults fed and deposited their eggs and on which the spring generation developed, have been greatly reduced in numbers on the plains and foothills through competition with red-stem filaree and other introduced pasture vegetation. The original native host plants of the beet leafhopper on the plains and foothills in California remain unknown up to the present time. However, to assume that the leafhopper changed its egg-laying habits from the perennial shrubby saltbushes to red-stem filaree in California does not seem plausible. As already stated, the perennial saltbushes were probably not the original host plants on which the spring generation developed but simply serve as food plants of the autumn generation during dry autumns.

According to Carter<sup>(10)</sup> the perennial plants were the principal hosts of the insect before the advent of the now widely scattered introductions, and through natural balance the predominance of the beet leafhopper was prevented.

## DISPERSAL AND MIGRATION

Such terms as "dispersal," and "migration" have in the past been used loosely and interchangeably by entomologists. Tutt<sup>(43)</sup> has pointed to the advisability of discriminating "between those movements which are made by insects from one part of their ordinary breeding grounds to another and those which make sudden and sweeping changes of location far outside of their natural breeding grounds." He has used "dis-

persal" for the former, and "migration" for the latter.<sup>4</sup> When insects migrate outside of their natural breeding areas, the migrants or their offspring are exterminated by unfavorable climatic conditions.

Accordingly, in this paper, *dispersal* of the beet leafhopper has been used for spring and autumn flights within the natural breeding areas, not resulting in extermination of the insect. Spring dispersals are the flights from the uncultivated plains and foothills into the cultivated areas, and the autumn dispersals are return flights from the cultivated areas to the uncultivated plains and foothills in California. *Migration* of the beet leafhopper has been used for flights outside of the natural breeding areas, resulting in the death of the offspring of the migrants.

Ball<sup>(2)</sup> suggests that the flights of the beet leafhopper "are in the nature of migration, northward in the spring and southward in the fall." There is no evidence to show that a return, autumn, southward migration from the migratory regions to the natural breeding areas occurs with the beet leafhopper in California.

#### SPRING DISPERSAL IN SAN JOAQUIN VALLEY

*Local Flights in Foothills.*—In years when the pasture vegetation did not dry too rapidly, an enormous congregation of spring-generation adults occurred near the San Joaquin entrance of Little Panoche Pass. At sunset the insects became active, flew higher than during the daytime and often came to rest on one's clothes or on the automobile, and the peculiar sexual behavior occurred as described in a previous paper.<sup>(29)</sup> The flights of the leafhoppers farther up the pass were toward the entrance, corresponding to the direction of the wind at sunset. There were years in which more rain fell near the summit than near the entrance of the pass and no congregations occurred toward the mouth of the pass. The adults were then found to be more abundant toward the summit (fig. 15). The leafhoppers prefer gravelly or rocky slopes in Little Panoche Pass, but when the pasture vegetation dries too rapidly in such localities they fly to green vegetation. It appears that the

<sup>4</sup> This use of the word "migration" differs from its use with birds, fishes, and certain other animals, where it is restricted to regular movements that constitute a racial custom with a hereditary basis and include a return journey either by the migrants or their offspring. (See, for example, the definition given by Thompson.<sup>(42)</sup>) Such movements rarely, if ever, occur with insects. Tutt,<sup>(43)</sup> who has assembled the facts bearing on the migration and dispersal of insects that have been recorded in the entomological literature during the last century or more, states: "As a matter of fact, it has never yet been thoroughly shown that the progeny of any [insect] immigrants, which have settled in new quarters, have returned to the home of their ancestors, although it has been considered highly probable in the case of certain locusts, and suggested also in the case of one butterfly, *Anosia archippus*."



struggle for favorable food is the stimulus which causes the insects to fly from one locality to another in this mountain pass.

*Into Cultivated Areas.*—The observations on the spring dispersal of the beet leafhoppers from the uncultivated plains and foothills into the cultivated areas of the San Joaquin Valley were made during the 1919 and 1925 outbreaks of the pest, and in years when the population was not at the maximum in numbers. Large flights from the foothills into the cultivated districts usually occur during warm, sultry, calm evenings. During the spring of 1919, three immense swarms of pale-green leafhoppers flew from the uncultivated plains and foothills into the cultivated regions of the San Joaquin Valley; one in the upper or southern part of the valley on April 8, another in the middle portion on April 14, and the third in the northern or lower section on April 28. It is not to be assumed that all of the leafhoppers acquired the winged stage and flew into the cultivated districts on the dates mentioned. In the middle part of the valley, nymphs and adults were still abundant on green patches of pasture vegetation on April 28. No nymphs were captured on dried pasture vegetation on May 14, and the adults were scarce on perennial plants. It is evident that later flights occurred, but after the invasion of the first large swarm into the cultivated regions, it is difficult to determine further movements from the plains and foothills, owing to flights which occur from unfavorable to favorable food plants within the cultivated regions.

During the summer an occasional adult was taken on various plants growing on the plains and foothills and in mountain passes (fig. 16), showing that not all of the leafhoppers fly into the cultivated areas during the spring dispersal.

In the southern section of the San Joaquin Valley, the beet fields were swarming with pale-green adults after the flight from the uncultivated plains and foothills had occurred. An examination of the vegetation in the Conner beet districts showed that there was a scarcity of annual saltbushes at the time that the invasion of the pest occurred, and this may account for the enormous congregation of the leafhoppers in the beet fields. During 1918, saltbushes made a normal growth, but in the spring of 1919, the saltbushes in the same localities, except in irrigated fields, made only a short growth and died, owing to a dry season.

When the immense swarms of beet leafhoppers flew into the cultivated regions on April 14, 1919, they were found during the next day generally distributed on green vegetation, but later they congregated on their most favorable host plants for the purpose of feeding and egg-laying. Large numbers of pale-green adults were found during the



Fig. 15. Summit of Panoche Pass, showing overgrazed hills sparsely covered with pasture vegetation, the most favorable habitat of the beet leafhopper.

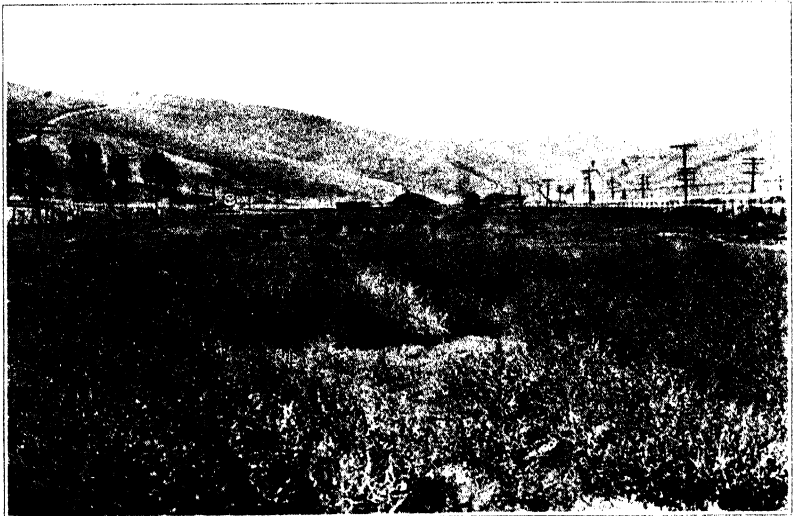


Fig. 16. Redscale or red orache (*Atriplex rosea* Linn.), growing in a mountain pass. After the spring dispersal the beet leafhoppers are generally distributed on annual saltbushes in the foothill regions, showing that not all of the leafhoppers fly into the cultivated areas.

spring on Russian thistles and different species of saltbushes, but these same plants were dry in July except in irrigated fields.

A few days were spent in the field to determine the northern boundary of the swarm. With the assistance of W. J. Hartung and the fieldmen of the Spreckels Sugar Company, the limit of the northern flight was found to follow roughly the Tuolumne River. The pale-green adults

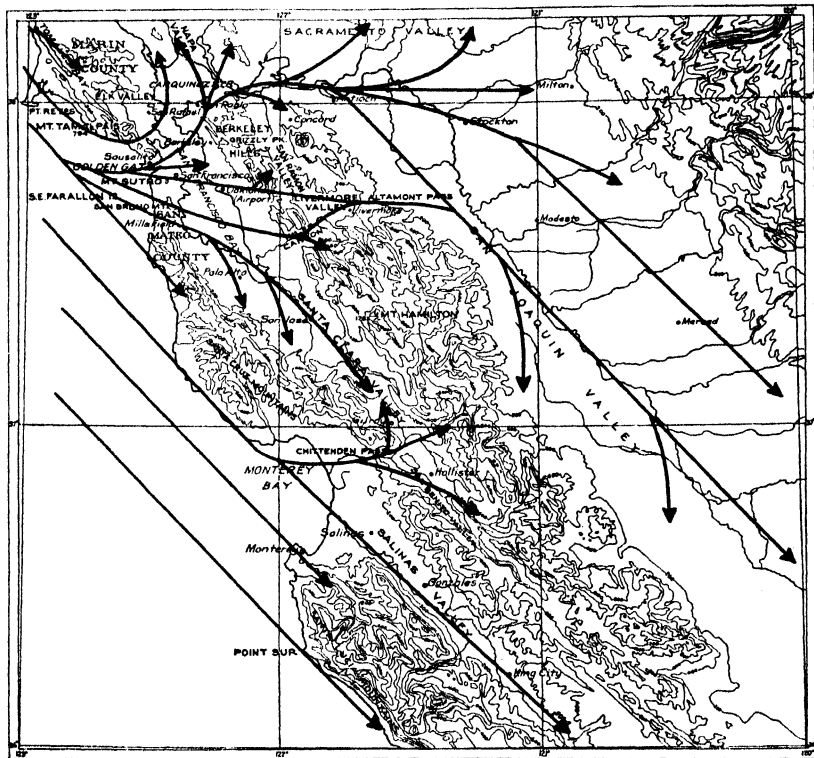


Fig. 17. Stream lines showing movement of air from sea over California.  
(From H. R. Byers.)

were taken in beet fields and on other host plants from the Coast Range to the foothills of the Sierra Nevada Mountains, a distance of about 50 miles.

A few pale-green adults were taken in the beet fields at Manteca on April 19, 1919, but they were very abundant in the northern San Joaquin Valley on April 28.

In years between outbreaks there was a gradual increase of the leafhoppers in beet fields and on favorable weeds in the northern San Joaquin Valley, indicating that a succession of northward flights occur in the cultivated areas sometimes after the pasture vegetation becomes dry

on the foothills. The prevailing winds in the San Joaquin Valley are northwest (fig. 17) and apparently the insects fly against light northwest winds during their succession of northward flights, although calm spells often prevail at sunset.

During 1922 Schwing<sup>(26)</sup> reported that the spring flights which occurred on April 21 from the Panoche hills extended as far north on April 23 as French Camp, a distance of about 85 miles.

According to Schwing,<sup>5</sup> a high population of beet leafhoppers occurred in the middle San Joaquin Valley during the spring of 1929 and were carried by northerly winds toward the Tehachapi Mountains, and hence a low population of the insects occurred in the Sacramento Valley. The winds were continually blowing from the north, northeast, and northwest during May, carrying the leafhoppers into the southern San Joaquin Valley.

#### SPRING MIGRATIONS

*Into Sacramento Valley.*—The appearance of the beet leafhopper in the Sacramento Valley is associated with a spring migration from the San Joaquin Valley. Fogs moving through Carquinez Strait may delay the migration of the leafhoppers into the Sacramento Valley. During the spring evenings a high fog frequently extends across the northern San Joaquin Valley. During foggy days the leafhoppers are sluggish and inactive, and when fogs occur before sunset no activity is displayed by the adults.

Schwing<sup>(27)</sup> found a gradual increase of the leafhopper on saltbushes in the northern San Joaquin Valley before, and a marked decrease after a migration into the Sacramento Valley.

The large migratory flights of the leafhopper into the Sacramento Valley usually occur in May, while the spring dispersal from the foothills into the cultivated areas of the northern and middle San Joaquin Valley occur in March or April. During the 1919 outbreak of the pest, the first large flights of the spring dispersals from the uncultivated plains and foothills into the cultivated regions of the San Joaquin Valley occurred on April 14 in the middle section and on April 28 in the northern section. In 1925 the spring dispersal was unusually early; the first flights occurred on March 29 and April 18, in the same parts of the valley.

Flights of small numbers of leafhoppers precede the large migration into the Sacramento Valley. The flights of the leafhopper during the 1925 outbreak, observed by Severin and Schwing<sup>(38)</sup> will serve as an

<sup>5</sup> E. A. Schwing, in a typewritten report to the Spreckels Sugar Company.

illustration. The first record of the spring migrants during 1925 was obtained in the inland beet fields of the Sacramento Valley on April 29. The leafhoppers were generally distributed but very scarce in the island or delta country on May 2. A large flight of the pest occurred on May 13, with variable winds from the south, southwest, and southeast blowing on May 11 to 13. No marked increase of the spring migrants occurred in the beet fields after May 22. The increase in the numbers of insects in the beet fields from May 14 to 22 was probably by short flights from unsuitable to favorable food and breeding plants. During 1925 Schwing observed a flight of the spring migrants in the beet fields near the city of Sacramento. The leafhoppers were flying everywhere and were settling on beets at dusk about 7:00 P.M. on May 13.

During 1922 to 1931 the maximum spring migrations into Sacramento Valley occurred on the following dates as determined by Schwing: 1922, May 25; 1923, about May 25; 1924, May 15; 1925, May 13; 1926, May 4; 1927, May 12; 1928, May 8; 1929, May 17; 1930, May 5; and 1931, April 26. In all of these years small migratory flights occurred before the maximum spring migration of the pest into the Sacramento Valley.

*Across Suisun Bay.*—During the 1925 outbreak, the beet leafhoppers probably flew across Suisun Bay to the beet fields near Suisun and Napa.

Lund<sup>6</sup> believed that the prevailing southwest winds from Suisun Bay blew the leafhoppers away from the island or delta regions toward the inland districts. It was assumed that the effectiveness of the winds against leafhopper invasion formed a triangle with the apex at Suisun Bay and the base extending roughly from Lisbon at the north, through Thornton and King Island at the south.

Carquinez Strait plays an important rôle in the direction of the wind. In the Sacramento Valley southerly winds prevail, but they are usually rather weak. The reason that Sacramento has southerly winds, while Stockton, 48 miles to the southward, has northwest winds, is that the former is north of Carquinez Strait and the latter south of it. The movement of the winds from Carquinez Strait is from the southwest toward the inland districts of the Sacramento Valley (fig. 17).

According to Mr. N. R. Taylor, of the United States Weather Bureau Office at Sacramento, the winds from Suisun Bay are of maximum velocity from 4 P.M. to midnight, decrease after midnight, and are of least velocity during the early morning hours. Calm days are rare in the Suisun Bay region during April and May, the months in which the migrations of the beet leafhopper into the Sacramento Valley occur.

<sup>6</sup> C. T. Lund, formerly Agriculturist of the Alameda Sugar Company, in a personal interview with the author.

When the leafhopper migrates northward from the San Joaquin to the Sacramento Valley, the relation of the direction of the wind in the San Joaquin Valley to calm days in the vicinity of Suisun Bay probably is important. Variable winds, such as northeast, south, or west, occur in the San Joaquin Valley when it is calm in the Suisun Bay. South winds, however, do not often blow in the San Joaquin Valley during April and May. When the sea breezes from San Francisco and Suisun bays strike the Sierra Nevadas, the winds are deflected to the north, creating a south wind in the Sacramento Valley, and to the southeast, resulting in a northwest wind in the San Joaquin Valley, and these winds prevail during the spring and summer.

Before the enormous hordes of leafhoppers migrated into the Sacramento Valley during the spring of 1925, Severin and Schwing<sup>(38)</sup> stated that there seemed to be no reason, with the information at hand from the Weather Bureau office, why it would not be possible for the leafhopper to invade the island or delta districts in large numbers during the spring migrations if conditions are favorable in certain years.

During the spring of 1925 a high population of beet leafhoppers occurred on the uncultivated plains and foothills in the middle San Joaquin Valley, but the pasture vegetation dried up rapidly in the southern part of the valley, and the population there was reduced. A severe outbreak of the pest occurred in cultivated areas of the San Joaquin and Sacramento valleys. During the spring of 1926 a high population occurred in the southern San Joaquin Valley and a low population in the middle section of the valley. A fairly low population of leafhoppers invaded the Sacramento Valley. It was assumed, therefore, that the origin of the pest in the Sacramento Valley is from the middle and northern San Joaquin Valley rather than from the southern part.

A peculiarity noted during the spring of 1926 was that a higher population of leafhoppers occurred on the Holland Land tract in the vicinity of Courtland than in any other locality in the Sacramento Valley. Fifty acres were plowed under owing to curly top on the Holland tract, but early-planted beets were not seriously affected by the disease. There were 600 acres of beets on Jersey Island, bordering the southern bank of the San Joaquin River, but the leafhoppers were extremely scarce in the beet fields. In the northern San Joaquin Valley the leafhoppers were abundant, and 50 acres of late-planted beets grown in Union Island were plowed under. It is difficult to explain the invasion of a high population of insects on the Holland Land tract by wind directions.

On April 14, 1921, the beet leafhoppers flew into the cultivated areas of the northern San Joaquin Valley and extended as far north as Wood-

land in the Sacramento Valley on April 15, but no specimens were taken in the Meridian beet fields about 30 miles north of Woodland on April 16, nor on April 30. A low population of leafhoppers occurs on the foothills from the Altamont Pass to the northern limit of the natural breeding grounds. If the flights into the cultivated areas started from the northern canyons in the San Joaquin Valley, the distance to Woodland would be approximately 60 miles. Leafhoppers have been taken on nettle-leaf goosefoot (*Chenopodium murale*) as far north as Red Bluff<sup>(28)</sup> in the northern extremity of the Sacramento Valley. The distance from the vicinity of the northern San Joaquin canyons to Red Bluff, probably covered in successive northward migrations following the cultivated areas, would be 150 miles.

There is no evidence to show that a return, autumn, southward migration of the beet leafhopper occurs from the Sacramento to the San Joaquin Valley. The slow southeastern flights parallel to the foothills at sunset in the San Joaquin Valley are movements within the natural breeding areas, and may be associated with the search for a favorable canyon or mountain pass or for food, or with mating or peculiar light reactions.

*Into Livermore Valley.*—It has often been suggested that the beet leafhoppers migrate from Corral Hollow through the Patterson Pass into Livermore Valley, situated between the Inner and Outer Coast ranges. Niles and Dublin canyons and the Altamont Pass open into this valley. Leafhoppers are scarce during the spring on the foothills bounding Altamont Pass and on the foothills toward the northern limit of the natural breeding grounds near Pittsburg. No nymphs have been found on the foothills bounding Livermore Valley.

*Into San Francisco Bay Districts.*—When a low population of the beet leafhoppers occurs in the natural breeding areas, curly top rarely occurs in the San Francisco Bay districts. During the spring of 1927 a migration of the pest into the San Francisco Bay districts occurred, probably from the San Joaquin Valley; in that year about 5 per cent of the tomatoes were infected with curly top in the districts east of the regions between San Francisco and Monterey bays. Tomatoes were also infected with curly top in the Hayward district during the 1919 and 1925 outbreaks of the leafhopper. During the spring of 1930 an occasional leafhopper was taken on common yellow mustard (*Brassica campestris*) near Pinole.

During the 1919 and 1925 outbreaks of the leafhopper, beets planted at Berkeley were all infected with curly top. Mangelwurzel, or stock beets, planted during April at Berkeley were also found to be naturally

infected with curly top.<sup>(87)</sup> During 1927, 1931, and 1932 the pest migrated into the fog belt of the San Francisco Bay district, and beets planted at Berkeley were again infected with curly top. An occasional leafhopper was taken east of the Berkeley hills during the spring of 1931. In all probability the flights of the leafhoppers were associated with southeast winds, spreading the insects into the San Francisco Bay districts from the San Joaquin Valley.

*Across San Pablo Bay.*—During the 1925 outbreak of the beet leafhopper the insects were present in the sugar-beet fields at Ignacio, crossing the San Pablo Bay in their spring migratory flights.

*Into Santa Clara Valley.*—Spring migrations of the beet leafhoppers occurred into the Santa Clara Valley during 1919, 1925, and 1927. Curly top of sugar beets was more severe towards the upper end of the valley. Tomatoes were also infected with curly top in these same years. In years between outbreaks an occasional curly top beet was found, indicating that a low population of the pest invades this valley. The leafhopper has been found breeding in rocky localities near Coyote and Lick in the Santa Clara Valley, but this is a minor and temporary breeding area. In years of abundance the insect probably migrates northward in the Santa Clara Valley from the foothills extending from the Santa Ana Valley to the western entrance of Panoche Pass.

*Into San Juan Valley.*—Schwing<sup>(26)</sup> reported that March and April plantings were destroyed by curly top on one side of the river in the San Juan Valley while on the opposite side of the river May plantings produced a good crop during 1921. The San Juan Valley is a continuation of the Santa Clara Valley, and the origin of the beet leafhopper in both valleys is probably the natural breeding area extending from Santa Ana to the western entrance of Panoche Pass. Migrations into both valleys probably occur from the San Joaquin Valley.

*Into Salinas Valley.*—It has been observed by Hartung<sup>(18)</sup> that when south winds blew in the Salinas Valley during April or May, the beet leafhoppers were found in the beet fields in the fog belt. He also noted that when winds blew from the east, southeast, or south, fogs were usually absent in this valley.

During the dry season the trade winds entering the Salinas Valley from Monterey Bay during the forenoon blow with increased force as the upper or narrow portion of the valley is approached. Northwest winds prevail throughout the entire season (fig. 17). Their maximum velocity is usually reached in the early afternoon, and as evening approaches they gradually decrease in force and nearly or quite cease during the night. The spring flights of the leafhopper from the foothills



into the cultivated areas probably occur during the evening or at night when the brisk winds cease blowing.

During the dry season the fogs arise from the sea, sweeping through Monterey Bay and encroaching upon the land very suddenly. The spring migrations into the fog belt from the upper end of the valley apparently are associated with southeast winds and occur when fogs are absent in the Salinas Valley.

The spring flight from the semiarid breeding centers to the fog-belt districts must be considered a migration. No return flights occur from the coastal regions to the natural breeding areas.

For many years the evidence was lacking that the leafhopper migrated from the San Joaquin into the Salinas Valley. The fact that the leafhoppers after the spring dispersal are found on favorable weeds growing along roadsides and in cultivated areas in mountain passes is no evidence that the insects migrate through the mountain passes. There used to be a large alkali sink near Bitterwater and another near Cholame covered with annual saltbushes on which the insects were found during the spring, summer, and autumn. When red-stem filaree became dry on the foothills the insects left the plants and assembled in the mountain passes on favorable food and breeding plants, on which the summer and autumn generations developed.

A sudden increase in the number of leafhoppers in the beet fields may be associated with a large flight from the foothills of the Salinas Valley. When the period of the spring dispersal from the natural breeding areas in the Salinas Valley is considered, and a concentration of leafhoppers in a valley from 7 to 9 miles in width and beet fields scattered from King City to Monterey Bay, a distance of 53 miles, a relatively low population on the foothills may result in a high population in the beet fields, especially outside of the fog belt.

During the period of 1918-1921, the first appearance of the pale green adults of the spring generation in the cultivated areas of the Salinas and San Joaquin valleys was as follows:

<i>Salinas Valley</i>	<i>San Joaquin Valley</i>
1918: May 8, King City	1918: April 24, upper
1919: April 2, King City	1919: April 8, 14, 28, upper, middle,
1920: April 22 to 23, King City	lower
1921: April 25 to 30, King City	1920: April 23, upper
	1921: April 6, 14, upper, middle

During the spring of 1920 the flights in the San Joaquin and Salinas valleys occurred at the same time, but during 1918, 1919, and 1921 the flights in the San Joaquin were earlier than in the Salinas Valley.

During the spring of 1922, Schwing<sup>7</sup> was convinced that the beet leafhoppers migrated from the San Joaquin into the Salinas Valley. The seasonal rainfall at the Spreckels ranch near King City was 13.15 inches<sup>8</sup> and the monthly rainfall was as follows: September, 0.15; October, 0.12; November, 0.28; December, 5.33; January, 3.36; February, 2.26; March, 1.09; April, 0.28; and May, 0.28 inches. The winter was unusually cold. He failed to find the leafhopper on the foothills near King City, San Ardo, Bradley, and Indian Valley and none were found on early-planted beets in the cultivated areas. A survey of the Panoche hills showed that few leafhoppers had acquired the winged stage on April 17, but in early May a high population occurred on these same hills. An enormous spring migration of leafhoppers must have occurred from the San Joaquin into the Salinas Valley on May 15; from 20 to 100 adults to 100 feet of beet row were estimated on May 16-17 in the beet fields near King City, Greenfield, Soledad, Gonzales, and Chualar.

During the spring of 1925 two large flights of the leafhoppers occurred in the Salinas Valley. The leafhoppers were abundant in the beet fields in the interior regions and fog belt of the Salinas Valley on March 27. As stated in a previous paper,<sup>(38)</sup> the early development of the spring generation was associated with a warm dry winter. The pasture vegetation became dry in January except on the north slopes of the eastern foothills in the upper Salinas Valley. The root system of red-stem filaree (fig. 18) was not destroyed by the drought, and this plant became green again after early February rains. A dry period occurred from February 22 to March 26, with a limited amount of rainfall—0.11 inches on March 9 and 10. The early flights in the Salinas Valley between March 24 and 26 were probably associated with the second drying of the pasture vegetation.

A second large flight occurred into the Salinas Valley on April 12, and the insects were also found in the San Felipe and Watsonville beet fields. After the second large flight occurred, a thorough search failed to reveal the presence of a single nymph on the eastern foothills bounding the Salinas Valley. Pale-green adults of the spring generation were found in all canyons and mountain passes examined from Chualar to San Miguel. In Chualar Canyon and Gloria Valley, situated in the fog belt, adults were taken, but no nymphs have ever been found in them. The fact that the adults were found on the foothills within the fog belt seems to indicate that a migration occurred from the San Joaquin Valley. In the mountain passes between King City to Bitterwater and

<sup>7</sup> E. A. Schwing, in a personal interview.

<sup>8</sup> According to the United States Department of Agriculture Weather Bureau station at King City, 12.12 inches was the seasonal rainfall.

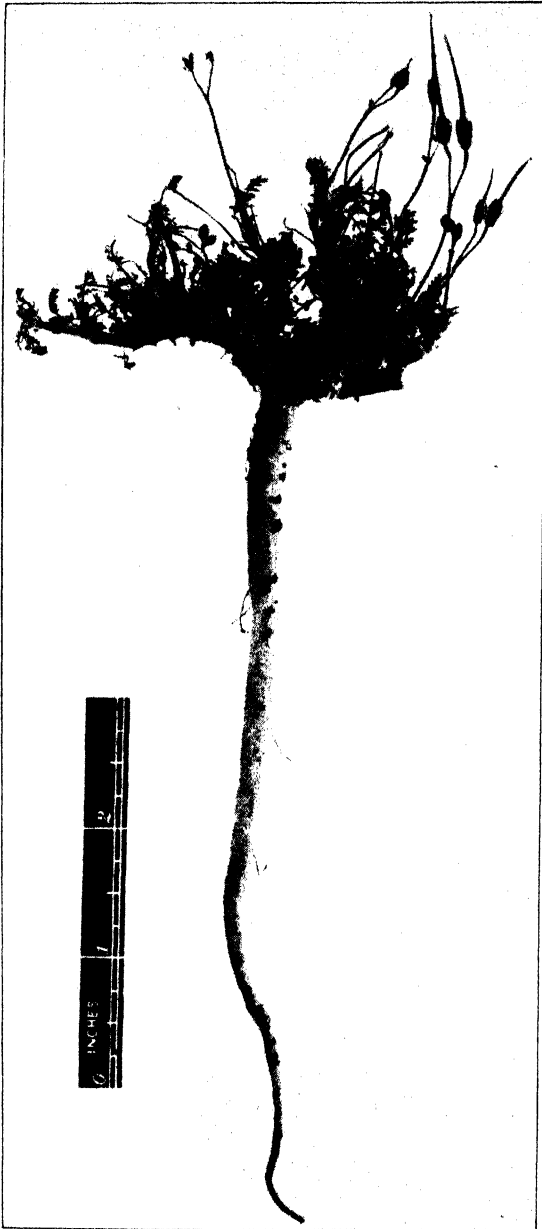


Fig. 18. Red-stem filaree (*Erodium cicutarium*) showing long taproot. During a dry spell in February and March the foliage becomes dry, cutting off the food supply of the nymphs, but after late spring rains, plants with long taproots may develop leaves again.

San Lucas to Coalinga, also in Reliz Canyon near Greenfield, and Pancho Creek near San Ardo, low-flying specimens were observed during the morning before the heavy winds began to blow.

Another trip was taken to the Salinas Valley on April 27-29. In Chualar Canyon only 7 adults were taken during one half day. The adults were still abundant in canyons and mountain passes outside the fog belt but no nymphs were found. Nymphs, however, were bred in the greenhouse from eggs deposited in red-stem filaree removed from canyons and mountain passes. Late spring rains fell during March, April, and May in the Salinas Valley, and the pasture vegetation which normally becomes dry during April or May remained green until June. A partial second brood developed on the foothills from eggs deposited by the migrating insects.

During the spring of 1927 the evidence indicated that the beet leafhoppers migrated from the San Joaquin into the Salinas Valley. The first warm days for several weeks occurred on April 20 and 21, and the pasture vegetation had dried considerably on the foothills in the middle San Joaquin Valley. Schwing observed, on April 21, short flights on the foothills in the vicinity of Coalinga, and during the daytime on the Cantua hills and in Big and Little Panoche passes. During the early evening on April 21 a light east wind was blowing which later changed to southeast.

According to Schwing,<sup>9</sup> the adults of the spring generation were not found on April 20 on favorable host plants growing in the cultivated areas near Manteca, Turlock, Merced, Chowchilla, and Fresno. During the afternoon and early evening on April 21, no leafhoppers were taken on saltbushes, Russian thistle, and lamb's quarters (*Chenopodium album*) from the Panoche hills to Merced, and east of Oro Loma. A return trip was made from Merced to Oro Loma on April 22, and the leafhoppers were taken on favorable weeds which had been swept during the previous day. An examination of Little and Big Panoche passes showed that a large flight had occurred from these mountain passes.

The leafhoppers flew with east winds from the San Joaquin into the Salinas Valley on April 21, 1927. Schwing sent the writer a telegram on April 21, stating that they were congregating in the entrance of Little Panoche Pass and that east winds would probably carry them into the Salinas Valley. The next morning leafhoppers were found basking in the sunshine on the upper surface of the beet leaves at King City. A very low population of the insects occurred on the eastern foothills bounding the Salinas Valley previous to the migration.

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<sup>9</sup> In a typewritten report to Spreckels Sugar Company.

There are two possibilities in regard to the paths of the migratory flights from the San Joaquin into the Salinas Valley: the leafhoppers flew either through the mountain passes or over the Inner Coast Range and the Gabilan Mountains. The general distribution of the leafhoppers in the canyons and mountain passes from Chualar Canyon to San Miguel during 1925 indicates that a mass movement of enormous hordes of leafhoppers from the uncultivated plains, canyons, and foothills in the San Joaquin Valley took place over the mountain ranges into the Salinas Valley.

*Into Southern Counties.*—During the 1919 outbreak of the beet leafhopper, spring migrations occurred in all of the principal beet centers located in the fog belt of San Luis Obispo, Santa Barbara, Ventura, Los Angeles, and Orange counties. In regions outside of the fog belt the insects were abundant and curly top was severe.

An outbreak of curly top occurred in the territory of the Union Sugar Company during 1925 and it was estimated that about 25 per cent of the crop was lost. The leafhoppers invaded the beet fields during 1922, 1926, and 1927 but no serious losses from curly top were sustained.

Although it was assumed that the leafhoppers migrated from the southern San Joaquin Valley into the coastal regions of these southern counties, no accurate mapping of other natural breeding areas has been attempted up to the present time.

*Method of Migration.*—According to Carter<sup>(9)</sup> "The appearance of the insect in areas separated from its natural breeding ground by high mountain ranges cannot readily be explained by a teleological theory of migration. The insect is extremely small and of light weight, and the slightest ground breeze will carry it for yards. It does, however, migrate, and should it encounter strong winds it is quite conceivable that it might be blown into upper air currents and by them be transported (in what must practically amount to cold storage, since these upper air currents are often of very low temperature) for long distances."

In California upper-air soundings have been made by airplane and in sounding balloon ascensions. There is conclusive evidence that along the coast there exists an upper stratum of warmer and relatively drier air. Air soundings have been made by airplane at the naval station on North Island, San Diego Bay, since January, 1923. The results of 35 afternoon flights during July and August, 1924, indicate according to Blake<sup>(3)</sup> that there is a decrease in temperature up to 500 meters, then an increase up to 1,250 meters, and another decrease parallel with the first, from that altitude upward (fig. 19). Blake writes:

As anticipated, the lowest temperatures were reached at the average level of the top of the clouds, the decrease for the first 500 meters averaging  $0.6^{\circ}\text{C}$  per 100 meters, or  $0.4^{\circ}\text{C}$  less than the adiabatic rate of dry air. From 500 to 1,250 meters a rise of  $7.2^{\circ}\text{C}$  is shown, and from 1,250 meters on the temperature fall is at the same rate as the initial decrease, or  $0.6^{\circ}\text{C}$  for each 100 meters. Surface temperatures corresponded generally with those found at 1,800 or 1,900 meters.

Naturally the relative humidity and temperature curves parallel each other rather closely but in an inverse sense [fig. 19].

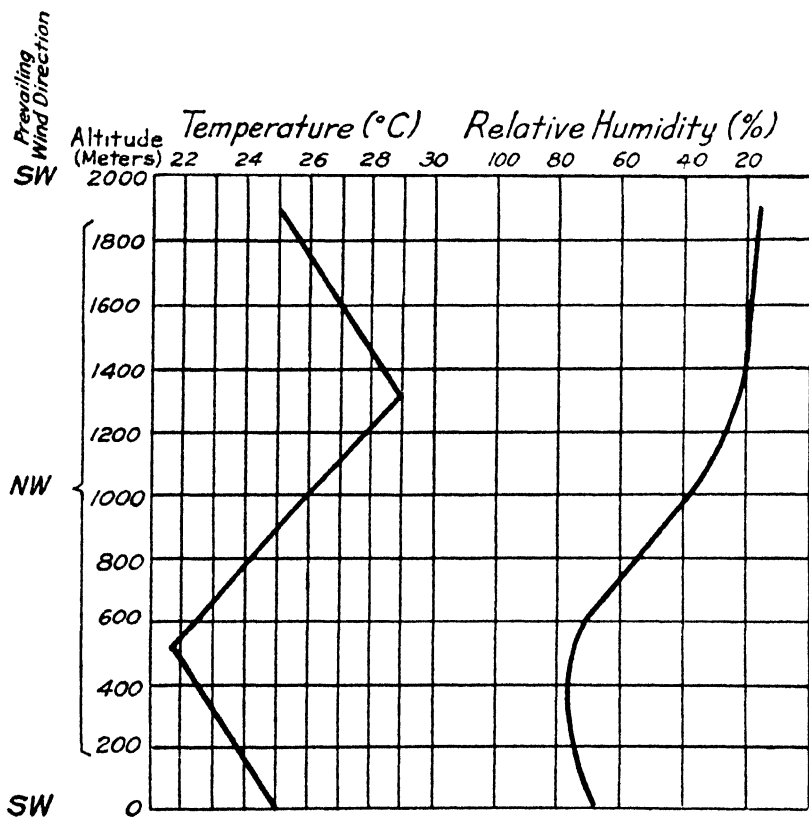


Fig. 19. Curve of average vertical temperature and relative humidity distribution during summer months over San Diego. (After D. Blake.)

Byers<sup>(6)</sup> in flying over the Santa Clara Valley and Monterey Bay during midafternoon at an altitude of 1,400 meters and in passing over the Santa Cruz Mountains experienced no cooling off. The descent to land at Oakland produced a sensation similar to stepping into a refrigerator. The temperature on land was  $17^{\circ}\text{C}$ , and a moist breeze was blowing at a moderate rate from the west.

Anderson<sup>11</sup> states that "during the summer the inversion ordinarily extends from southern Washington to a point 800 to 1,000 miles south of San Diego, west from the coast for a distance of 200 to 300 miles, and over the land areas between the ocean and the Coastal Range."

Blake<sup>10</sup> states:

The eastern limit of the summer temperature inversion here in the south is not fixed, and the extent of the stratum varies not only with topography, but also with the changes that take place in the controlling weather factors. At times the cloud layer, which is used as an index to the extent of the stratum of cooler air from the ocean, is found a short distance only over the land, while again it penetrates some distance over the valley into the mountains.

I think that it may be assumed that in central California the inversion will be found usually extending to the Coast Range, and at times penetrating through the passes you have mentioned [Pacheco, Little and Big Panoche, Coalinga—King City, and Cholame].

Beyond the Coast Range, the descending currents on the lee side give rise to dynamical heating and the inversion is not possible. For this reason it is almost certain that the inversion common to the coast will not be found as such as far inland as Stockton, but might reach at times to Antioch.

Byers<sup>11</sup> wrote as follows:

Your question concerning the vertical temperature distribution above the San Joaquin entrance to various passes in the Coast Range is almost impossible to answer because free air soundings are completely lacking in that region.

Surface observations from that locality and also observations from Mount Hamilton and a few observations I have made in flying over this region indicate that during most of the day there is no well-defined inversion over the San Joaquin entrances, although it is usually well marked during the morning and night at the western ends of these passes. In the late fall, winter, and early spring during periods when there is no cyclonic activity, very strong inversions build up in all the valleys and canyons of the Coast Ranges and in the San Joaquin Valley as well. At this time there is no sea breeze, but the air is practically calm at the surface with a movement from east to west at higher levels. On numerous occasions temperatures of 50 to 60° F have been observed at Mount Hamilton when in the valleys all around it was near freezing.

The spring flights of the beet leafhopper have frequently been observed at sunset during calm evenings in the eastern entrance of Little Panoche Pass. The flights could be followed out of the entrance of Little Panoche Pass at sunset, but the flight of an enormous swarm out of the pass has never been observed. The flights appeared to be associated with ascending air currents at dusk. The ascending air currents

<sup>10</sup> Blake, D., letter to the author dated February 15, 1932, in response to a question as to whether he believed temperature inversion occurred above the San Joaquin entrance of certain passes.

<sup>11</sup> Byers, H. R., letter to author dated February 16, 1932.

may carry some of the leafhoppers into the upper air stratum and here they may drift and fly long distances, possibly over mountain ranges. Their presence in the upper air could be determined by airplanes equipped with insect-collecting traps carried between the wings.<sup>(44)</sup>

According to Byers, a movement of the higher air from east to west occurs during early spring, when there is no cyclonic activity and the air is practically calm at the surface, and the leafhopper could readily be transported by the higher winds from the San Joaquin Valley into the Salinas Valley.

The following examples of migrations of the beet leafhopper in other states and islands may represent transportation of the insect by upper air currents rather than by surface migrations through successive flights from the natural breeding areas.

Ball<sup>(2)</sup> has found the beet leafhopper in abundance on the snow on Pikes Peak above 14,000 feet and has captured examples on the Beaver Mountains at 12,000 feet. They were swarming near Panguitch, Utah, at an elevation of 7,000 feet, just at the time the immense swarms swept over the beet regions of Utah in 1915.

Whirlwinds may have carried the leafhopper to these high altitudes or the insects may have been transported by the higher winds.

During 1926 Carter<sup>(9)</sup> found the beet leafhopper on the east side of the Continental Divide in Montana and a reasonable interpretation was that these specimens represent the few straggling individuals which had survived a long-distance migration. Migrations from the breeding grounds in Idaho and Washington probably occur into the Bitterroot Valley and Flathead (Kalispell) districts of Montana.

Severin and Severin<sup>(39)</sup> found that curly top of sugar beets occurred on rare occasions in the beet fields of the west-central part of South Dakota. Sweepings with an insect net from the most favorable host plants of the beet leafhopper failed to include a single specimen. It was demonstrated, however, that previously noninfective beet leafhoppers transmitted curly top from South Dakota diseased beets to healthy beet seedlings under greenhouse conditions in California. If the beet leafhopper was responsible for the diseased beets which were found in South Dakota, there is a possibility that the insects migrated into west-central South Dakota from regions outside of the state.

According to Ball<sup>(2)</sup> curly top of sugar beets has been reported from Nebraska and Kansas. In all probability Kansas and Nebraska are outside of the range of its natural breeding grounds and the beet leafhopper migrated into these states.



Carter<sup>(10)</sup> reported tomato curly top transmitted by the beet leafhopper in Muscatine, Iowa, as probably a case where the insect migrated out of its normal range into areas where it cannot survive.

De Long<sup>(12)</sup> collected nymphs and adults of the beet leafhopper in abundant numbers at Miami, Florida, on sea purslane (*Sesuvium portuacastrum* L.) growing natively on the upper beach and sand dunes of the bays and inlets of the Atlantic Coast. They were collected during April, 1921. Ball visited the same localities in Florida in later years but failed to collect the leafhopper. Whenever the leafhopper migrated into the coastal regions of California it failed to establish itself. In all probability the leafhopper migrated to Miami, Florida, from the southwestern part of the United States or northern Mexico.

Henderson<sup>(20)</sup> failed to find the beet leafhopper on Isla Raza on May 16, 1928, whereas Van Duzee<sup>(45)</sup> captured many specimens on April 21 and May 4, 1921. A migration of the pest probably occurred from Lower California to Isla Raza during 1921.

#### SUMMER DISPERSAL IN SACRAMENTO VALLEY

An attempt was made to determine the longest distance that the adults of the summer generation flew to reach the late-planted beet fields in the Sacramento Valley during 1925. In many fields of unthinned and thinned beets examined in June, only a low percentage of curly top was present, owing to the fact that these beets had germinated after the flights of the spring migrants had ceased. The nymphs of the summer generation began to acquire the winged stage about July 1, and a gradual increase of the adults occurred in late May, June, and July plantings. One field of 100 acres of unthinned beets contained at least one leafhopper to each beet on July 14. There were no other beet fields within 15 miles on the north, east, or south; the nearest beet field was 3 miles to the west. The adults of the summer generation must, therefore, have flown at least 3 miles to the 100 acres of unthinned beets, although some of them probably invaded the late-planted beet fields from weeds growing in the vicinity. The flights may have been influenced by the prevailing winds from Suisun Bay. In all probability the flights of the adults of the summer generation from one beet field to another was associated with a food stimulus. An examination of the beet fields planted in March, April, and May showed that many of the beets had died owing to curly top. Extremely hot weather had scorched the outer leaves of the beets leaving a tuft of diseased, thick, leathery leaves.

## SUMMER MIGRATION FROM SAN JOAQUIN VALLEY

During the 1919 outbreak of the beet leafhopper, summer migrations from the San Joaquin Valley occurred. During the spring thousands of leafhoppers were found on various species of saltbushes in the southern San Joaquin Valley, but in July it was difficult to secure 100 adults on these same plants. In the Connor and Corcoran beet districts the sugar beets were mostly dead owing to curly top, but the adults were scarce on the green innermost leaves with dried outer foliage of such beets as could be found. Sweepings were made on some of the most favorable host plants, such as the saltbushes growing in and along the margin of beet fields, but the leafhoppers had not assembled on these plants.

In the middle San Joaquin the beet leafhoppers were extremely abundant on Russian thistles and various saltbushes during April, but in July the adults were rare on these plants. Along the roadsides and in the fields that had not been irrigated, Russian thistles attained a few inches of growth and died during the spring owing to a shortage of rain and probably also to the drain of enormous hordes of leafhoppers.

In the northern San Joaquin enormous numbers of adults had congregated on June 26 on *Atriplex bracteosa* growing among diseased beets in the vicinity of Hatch Station. The foliage of these saltbushes was covered with droplets of clear excrement which glistened in the sunshine. When a person walked past one of the weeds, so that a shadow was thrown on the plant, a swarm of leafhoppers flew up. Nymphs were still abundant on sugar beets with green innermost leaves and dried outer foliage. The next visit to these beet fields on July 5 showed that most of the insects had left the saltbushes and that a summer migration had occurred. Another assemblage of leafhoppers was observed on *A. bracteosa* on July 26.

## AUTUMN DISPERSAL

*In San Joaquin Valley.*—The earliest record of the return flights to the foothills of the Coast Range in the northern San Joaquin Valley was on October 8, 1919, before the pasture vegetation germinated. During 1919 the plants and foothills were not covered with green pasture vegetation until after the rains fell on December 1 to 6. A few adults were taken in each sweeping on Bermuda grass (*Cynodon dactylon*) growing along a creek in a canyon. An occasional specimen was taken on tarweed (*Hemizonia virgata*) and on *Eriogonum angulosum*.

In the middle San Joaquin Valley the adults were captured during October, 1919, on perennial plants growing on the uncultivated plains and in canyons and mountain passes. The insects were also taken in large depressions and squirrel holes on the plains, where the insects probably sought the shade.

During November, 1919, the autumn dispersal of the leafhoppers to the foothills was at its maximum in the northern and middle San Joaquin Valley. The insects were most abundant on perennial plants growing in canyons and mountain passes. In canyons perennials were usually abundant in dry streamways, tributaries, and drainage furrows. Specimens were also taken on tree tobacco (*Nicotiana glauca*) and pepper trees (*Schinus molle*).

An attempt was made to study the movements of the leafhopper near and in the entrances of canyons in the northern San Joaquin Valley, but only an occasional specimen was captured flying south about 3 to 4 feet above the surface of the ground. The windshield of an automobile facing the setting sun attracted dozens of adults, which exhibited a peculiar sexual behavior.<sup>(20)</sup> The automobile was stationed about  $\frac{1}{2}$  to 1 mile outside the mouth of various canyons, and in every case the leafhoppers settled on the machine in a few minutes. Several miles from the foothills, the leafhoppers were attracted to the auto in stubble fields, plowed fields, and in a graveled road between two plowed fields where no green food was available. The reflected light rays from the setting sun on the windshield may have attracted either the higher-flying insects moving from the cultivated areas to the foothills, or possibly those which had settled on the ground before reaching the foothills, since low-flying specimens were rarely taken on the wing.

In the northern San Joaquin Valley cultivation often extends to the base of the foothills and occasionally to the hills; hence a more favorable locality to study the flights was found in the middle section of the valley, where the plains and foothills are covered with pasture vegetation. Whenever the automobile was stopped on the plains so that the windshield faced the setting sun, the adults assembled on the car and the pairing of the sexes was observed. By sweeping with an insect net an occasional specimen was captured in the dry pasture vegetation growing on the plains. When the hillsides became partly shaded, dozens of low-flying leafhoppers could be plainly seen slowly flying southeast down the valley following the general direction of the foothills. No similar movement of leafhoppers was observed on the plains (fig. 20, *P*) from  $\frac{1}{4}$  to 3 miles away from the foothills nor in the cultivated areas. The flying leafhoppers appeared white in color and hence could be dis-

tinguished easily from the multitude of other insects hovering in the air at sunset. In the sunshine they became invisible about 15 feet above the surface of the soil where a background was lacking.

The flights of the leafhoppers were also studied on November 11 to 13, 1919, in Wild Cat Canyon, situated about 5 miles west of Oro Loma, where they were extremely abundant. When the sun warmed the foothills occasional specimens were observed on the wing at about 11 A.M.

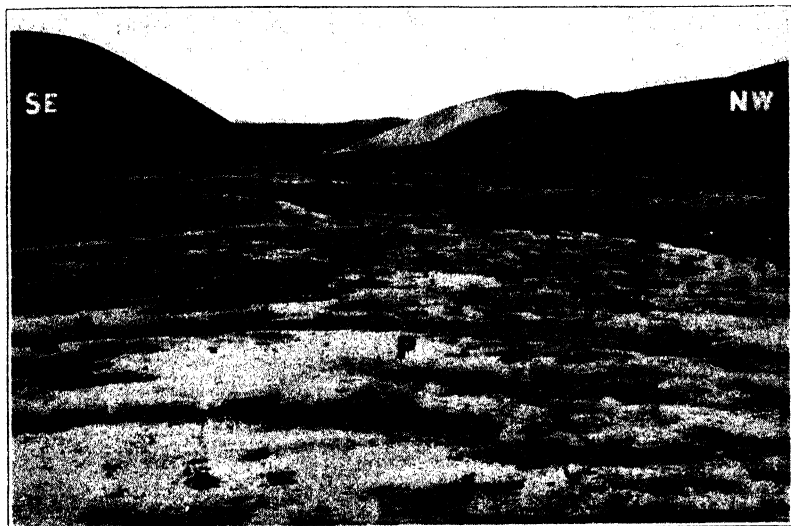


Fig. 20. Wild Cat Canyon, 5 miles west of Oro Loma. SE, southeast foothill; NW, northwest foothill; P, plains, showing perennials which serve as food plants of the beet leafhopper during dry autumns, before the pasture vegetation has germinated. After the pasture vegetation germinates the beet leafhoppers leave the perennials and are found on red-stem filaree (*Erodium cicutarium*) growing on the plains and foothills.

in the mouth of the canyon, where they flitted about here and there, probably in search of food. At 4:30 P.M. the adults were common in the air, and at 5 P.M. the activity was at its height and continued until shortly after sundown. The flights of the insects could be seen across a dry creek with the southeastern foothill (fig. 20, SE) bounding the entrance of the canyon as a background. Some of the leafhoppers flew across the sunny canyon, and when they entered the shadow of the southeastern foothill, they settled to the ground; others flying higher continued southeast along the Coast Range. The foothills situated on the southeastern side of the entrance to the canyon were higher and projected out farther than the northwest hill (fig. 20, NW), and as the sun was sinking behind the mountains the former became shaded sooner

and the latter cast a larger shadow on the plains. An examination of the southeastern foothills showed that the leafhoppers were abundant in dry pasture vegetation and on perennial shrubs, on which the sexual behavior was observed. A striking peculiarity was the fact that they were common on dry pulverized soil along the steep banks of the creek. Enormous numbers had assembled on *Atriplex polycarpa* growing on the banks and bottom of the creek. Investigations were made 1 mile up the canyon, and the same relative abundance was found on different species of plants. The leafhoppers were frequently observed flying south in the sunny canyons.

The leafhoppers do not fly in the vicinity of the foothills or canyons on cloudy days or when heavy winds are blowing. On November 18, the sky was overcast by dark clouds at 4 P.M., and it remained cloudy until near sunset, when it cleared for a few minutes at intervals. When the sun was shining, the canyon soon became warm, and occasional leafhoppers were observed on the wing. They were attracted to the automobile, but when the sun was hidden behind the clouds, the temperature dropped and they settled to the ground.

During the autumn dissemination a southward movement toward the foothills was observed by an occasional low-flying leafhopper. Specimens attracted to the automobile were captured during the autumn flights in the cultivated areas, and when they were liberated the general direction of flight was southerly towards the foothills in the northern part of the valley. The insects were set free during an apparently calm spell, but when a handful of dust was thrown into the air, the compass indicated that the particles were carried in a southward direction. Similar tests were made on the plains in the middle of the valley; there the insects flew south when a north breeze was blowing, or southeast with a northwest breeze.

During the autumn leafhoppers fly into the eastern and western entrances of mountain passes in the Inner Coast Range. In the autumn of 1919, they were found in the eastern and western entrances of the Altamont and Cholame passes. In the autumn of 1925 they were commonly taken in the western entrance of Panoche Pass near Paicines. In the spring of 1925 and 1926 nymphs were abundant for a distance of about 5 miles in the western entrance of Panoche Pass.

During October and November the activity of the leafhoppers is probably aroused by a lowering of the temperature at sunset, but during December flights have been observed during the morning and afternoon. During the autumn they have frequently been observed flying against light breezes in canyons and mountain passes. A study of the flights

at the mouth of canyons indicates that air currents blowing out of the canyons at sunset are probably not the only stimulus which causes the leafhoppers to fly into the canyons, because many of the insects flew past the entrance and followed the contour of the hills or settled to the ground when they entered the shade. Light reactions at sunset may also play a role in guiding them into canyons and mountain passes. The windshield of an automobile attracted hundreds of leafhoppers at sunset; they resembled the swarming of enormous numbers of insects around an electric arc lamp.<sup>(29)</sup>

*In Sacramento Valley.*—An autumn dispersal of the beet leafhoppers from the cultivated areas to the foothills of the Inner Coast Range occurs in the Sacramento Valley. In years when an outbreak of the pest did not occur, a low population of dark overwintering adults were found in small valleys and canyons located in the eastern foothills of the Coast Range bounding the western side of the Sacramento Valley. In the southern part of the Sacramento Valley, the Montezuma and Yolo hills are mostly cultivated, and the foothills west of Winters are covered with orchards and are not favorable winter quarters for the leafhopper. After the 1925 outbreak of the pest, the insects were taken on red-stem filaree and weeds growing on the foothills of Vaca and Capay valleys and in canyons west of Dunnigan, Williams, Maxwell, and Willows. The dark overwintering forms were also taken on red-stem filaree growing on the west side of the Marysville Buttes.

An investigation was also conducted on the foothills of the Sierra Nevada bordering the eastern side of the Sacramento Valley. The foothills are often rolling or merely undulating, and the timbered region is soon reached after leaving the valley slopes. The leafhopper was rarely taken during the autumn on the foothills. Red-stem filaree is not abundant on the hills and there is no indication on this side of typical beet-leafhopper foothill breeding grounds. The locations investigated in the Sierra Nevada foothills were: 7 miles northeast of Red Bluff; 10 miles east of Chico; vicinity of Oroville; 12 miles east of Marysville; vicinity of Newcastle; and vicinity of Lone.

*In Salinas Valley.*—In the Salinas Valley the return flights of the dark overwintering adults follow the Salinas River and its tributaries flowing from the Gabilan Mountains bounding the east side of the valley. With the approach of the rainy season the trade winds gradually decrease in force. During November there are occasional days with calm evenings when the autumn flights of the leafhopper to the foothills have been observed. Short flights from perennial to perennial were observed in the canyons. The movement of the leafhoppers from salt-

bushes covering a large alkali sink in a mountain pass at Bitterwater was not eastward toward the Inner Coast Range but westward into the Gabilan Mountains.

A blanket of fog usually covers the base of the foothills of the Sierra Santa Lucia Range as far south as Greenfield, a factor which is unfavorable to the overwintering adults.

#### STIMULI OF DISPERSAL AND MIGRATION

The spring-dispersal flights of the beet leafhopper are probably associated with a food stimulus; the adults fly from the uncultivated plains and foothills after the pasture vegetation becomes dry and invade the cultivated areas when the annual saltbushes are succulent and most favorable from the standpoint of food and egg deposition. The autumn flights of the leafhopper are probably also associated with a food stimulus; the insects fly from the cultivated territory when the saltbushes and other favorable weeds become woody and dry. In the Salinas Valley, however, the dark overwintering adults left large beets and even small beets in experimental plantings during 1919, indicating that the autumn flights will occur regardless of whether favorable food is available or not.

There does not seem to be any consistent relation between reproductive stimuli and the spring and autumn dispersals of the leafhopper. After the spring dispersal about 8 per cent of the spring-generation adults that invaded the cultivated areas in the middle San Joaquin Valley were males and 92 per cent females. Most of the males remain behind on the uncultivated plains and foothills and are common on perennials after the pasture vegetation is dry, but are rarely taken during the summer and probably die. Dissections of females after the first spring flight into the cultivated areas had occurred showed that 92 per cent had mature eggs in the ovaries. During the autumn flights, however, most of the males follow the females to the uncultivated plains and foothills, and mating occurs during the autumn. The dark overwintering females are not at the egg-laying stage during the autumn dispersal; dissections of specimens collected in canyons of the Coast Range in the northern and middle portions of the San Joaquin Valley at intervals showed the following average percentage at the egg-laying stage: December, 4 per cent; January, 52 to 64 per cent; and February, 86 to 99 per cent. The males die during the winter. It is difficult to associate the mating and egg-laying stimuli with the spring and autumn flights of the leafhopper.

The stimulus for summer migrations of the adults is probably hunger, owing to overcrowding. As already mentioned, the sugar beets in the southern section of the San Joaquin Valley in 1919 were mostly dead as a result of curly top. The most favorable host plants were stunted and dry, except in irrigated fields, and yet the adults had not assembled on these plants in irrigated fields. The leafhoppers, however, will leave green succulent plants in the Imperial Valley, without an apparent stimulus as reported in previous papers.<sup>(28, 30)</sup>

### NATURAL BARRIERS

*In San Joaquin Valley.*—During the autumn dispersal of the beet leafhoppers from the cultivated areas to the foothills, large numbers of adults often congregate during dry autumns on perennials growing in canyons in the northern San Joaquin Valley. When the populations of leafhoppers during the autumn and spring on the northern foothills and in canyons were compared, it was evident that a marked reduction of the spring generation occurred during a normal season of rainfall. After the 1919 and 1925 outbreaks of the pest a marked decrease in population of the spring generation occurred compared with the autumn generation in the Altamont and Pacheco passes.

In the San Joaquin Valley rainfall decreases from north to south, and with minor exceptions is considerably less on the western side of the valley than on the eastern side. Stockton, in the northern part of the San Joaquin Valley, has an annual rainfall of 14.35 inches, while Bakersfield, in the southern part of the valley, has 5.68 inches. Table 3 gives the annual rainfall of towns on the western and eastern sides of the valley.

Heavy rainfall kills some of the leafhoppers. At Manteca  $3\frac{3}{4}$  inches of rain fell on September 11 to 13, 1918, before the return flight of the insects to the uncultivated plains and foothills had begun. In sugar-beet fields dead adults with wings spread were observed partly embedded in sandy soil below the leaves of diseased sugar beets and also in the folds of dried beet leaves. Dead leafhoppers partly embedded in the soil were also commonly taken below branches of *Atriplex bracteosa*, where they evidently crawled to escape from the rain. Dead nymphs were rarely found, but if present they would probably have been difficult to detect. An examination of the adults under a binocular microscope showed that 50 per cent had been parasitized. The material was dry and could not be dissected to determine whether the remaining 50 per cent were not weakened forms that had parasitic larvae within their



bodies. Leafhoppers near the end of their natural life often become sluggish and inactive, and of all of the dead leafhoppers taken only one dark overwintering adult was found; the remainder were adults of the summer generation.

In the canyons and mountain passes of the northern San Joaquin Valley there are limiting factors which check the multiplication of the

TABLE 3  
AVERAGE ANNUAL RAINFALL FROM NORTH TO SOUTH IN SACRAMENTO  
AND SAN JOAQUIN VALLEYS

Western half		Eastern half	
Station	Rainfall, in inches	Station	Rainfall, in inches
Sacramento Valley			
Red Bluff.....	24.81	Chico.....	23.60
Corning.....	21.36	Oroville.....	27.50
Willows.....	16.35	Marysville.....	19.60
Colusa.....	15.95	Sacramento.....	17.96
Woodland.....	17.88		
Vacaville.....	26.53		
San Joaquin Valley			
Antioch.....	12.68	Milton.....	21.31
Tracy.....	10.58	La Grange.....	16.91
Westley.....	10.65	Lemon Cove.....	15.14
Newman.....	11.17	Porterville.....	10.02
Los Banos.....	8.10		
Dos Palos.....	8.29		
Mendota (near valley trough).....	5.88		
Coalinga.....	7.24		
Dudley.....	7.07		
Lost Hills.....	5.72		
Middlewater.....	6.40		
Maricopa.....	6.36		

leafhopper. Porterville, with an annual rainfall of 10.02 inches, is near the northern limit of the natural breeding area in the Sierra Nevada foothills, on which a low population of insects occurs. Coalinga, situated among the foothills on the western side of the valley, has an annual rainfall of 7.24 inches, and conditions are favorable for the multiplication of the leafhopper. Bakersfield, located in the plains district of Kern County, has an annual rainfall of 5.68 inches, and most of this county is favorable for the insect.

It has frequently been observed that when red-stem filaree grows tall and dense the leafhoppers leave the vegetation. They prefer short filaree on barren hillsides.

The character of red-stem filaree may be an indicator of favorable foothill breeding grounds, but, nevertheless, there may be composite controlling factors which hold this insect in check in some of the canyons and mountain passes of the northern San Joaquin Valley, even where short red-stem filaree occurs. The character of the day from sunrise to sunset is different in the northern part of the San Joaquin Valley from that in the middle and southern sections of the valley—there are more cloudy and partly cloudy days. The northern section of the valley has a lower percentage of sunshine than the middle and southern portions. Fogs moving through Carquinez Strait and spreading over the northern San Joaquin Valley may be another factor in the complex climatic barrier that destroys the overwintering adults.

Exposure and shading of hills is an important factor in the habitat of the overwintering insects. The leafhoppers prefer the south exposure on the hillside when food conditions are favorable, but when the pasture vegetation becomes dry they are sometimes found abundantly on the north slopes.

The leafhoppers prefer gravelly or rocky slopes to soil which holds the moisture. During the spring the nymphs are often seen basking in the sunshine on the warm stones. Frequently the insects are abundant on squirrel mounds.

A critical factor in the reduction of the spring population in the northern San Joaquin Valley may be associated with the hatching of the nymphs. At Manteca eggs deposited in the foliage of sugar beets from November 1 to January 15, 1919, failed to hatch out of doors. It was commonly observed in the greenhouse that eggs will push out of the slit-like egg chamber, but the nymphs often failed to rupture the chorion or died in the process of extrication from the eggs, and this may be associated with humidity and temperature.

*In Sacramento Valley.*—Rainfall is an important exterminating factor of the beet leafhopper in the Sacramento Valley. The rainfall increases northward in the Sacramento Valley and varies from 17.96 inches at Sacramento near the southern boundary of the valley to 24.81 inches at Red Bluff in the northern extremity. The precipitation is considerably less upon the western side of the valley than in corresponding localities upon the eastern side. The rainfall along the western side decreases from the south to about the central part of the valley and then increases to Red Bluff. The rainfall along the eastern side increases from south to north throughout the valley. Table 3 shows the average rainfall from north to south at the weather bureau stations situated in the western and eastern halves of the valley.

The winter humidity is high owing to rains and fogs in the Sacramento Valley. A low atmospheric humidity accompanied by cloudless skies is usual throughout the summer, and is favorable for the spring migrants and summer progeny of the leafhopper. In the southern portion of the valley the relative humidity is about 10 per cent higher than in the northern part.

Fog is common during the winter months, but decreases in density and frequency of occurrence northward in the Sacramento Valley. In the southern part of the valley, fog is dense during the night and morning, but frequently disappears or lifts during the day, though sometimes it continues as a high fog for several days. The lower-lying parts of the valley are sometimes subject to light fogs in the autumn and spring, when other portions are free from it.

After the serious outbreak of the leafhopper during 1925, adults were found during the autumn in canyons of the Coast Range and in the cultivated areas. Food for the insects had been favorable in the canyons since the October rains had germinated the seeds of red-stem filaree. In the cultivated areas adults were taken during the autumn on spearscale (*Atriplex patula*) (fig. 21) growing along irrigation ditches. During the winter, however, the adults were exterminated in both the canyons and cultivated areas. The exterminating factor was the dense ground fogs which occurred daily from December 22 to January 18. During 28 days of this fog period there were only 4 hours of sunshine. Rainfall apparently was not the direct exterminating factor during the fog period. The precipitation during the 28 days of fog at Sacramento was as follows: January 2, 0.02; 14, 0.03; 16, 0.01; 18, 0.15; total, 0.21 inches.

Another factor unfavorable to the overwintering adults may be the heavy dew which occurs during the rainy period in the Sacramento Valley.

The Sacramento Valley has a lower percentage of sunshine than the middle and southern parts of the San Joaquin Valley.

In the cultivated areas of the Sacramento Valley the most favorable breeding plants, such as the saltbushes, are scarce, except in the southern part of the valley. But red-stem filaree is abundant on the foothills of the Coast Range and on the west side of the Marysville Buttes, and hence food and breeding plants are not the limiting factors which prevent the leafhopper from establishing itself in the Sacramento Valley.

After the beets were harvested in the Sacramento Valley during the 1925 outbreak of the beet leafhopper, large numbers of leafhoppers flew into adjacent bean fields, and a high mortality of the insects occurred.

Dead leafhoppers with their mouth parts inserted in the tissues of the leaves were common on pink beans. In all probability a high mortality of the nymphs occurs after the beets are harvested and the tops become dry, although large numbers of leafhoppers were found on

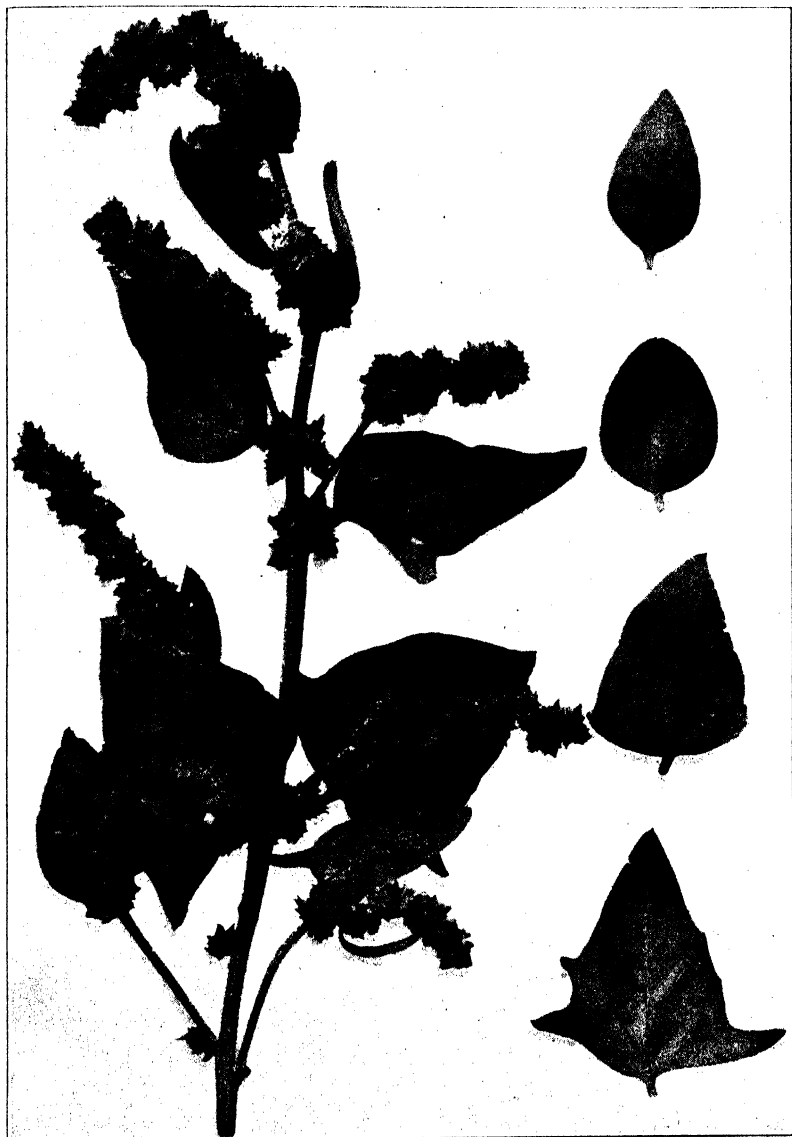


Fig. 21. Branch of spearscale (*Atriplex patula*) showing leaves and fruiting bracts, also different-shaped leaves removed from plant.

*Atriplex patula* growing along irrigation ditches after the beets were harvested.

When insects migrate from their natural breeding grounds, they fail to establish themselves in their new environment unless they encounter conditions similar to their original habitat. When the migrating swarms of the beet leafhoppers invade the beet fields under new conditions of environment, barriers rarely affect the migrants, which usually destroy the beet crop when they are at their maximum in numbers. The hot, dry summers in the Sacramento Valley are favorable to the migrants and later generations in the cultivated areas, but the overwintering progeny is exterminated by the winter conditions.

*In Salinas Valley.*—Rainfall above normal is a factor which reduces the beet leafhopper in its natural breeding areas in the Salinas Valley. There is not only a wide variation in the rainfall of the Salinas Valley from year to year, but often considerable variation for the same season in different parts of the valley. The rainfall diminishes as the head end of the valley is approached, although this is subject to variation, and the seasonal rainfall at Soledad averages less than at either Salinas or King City. The seasonal rainfall may vary from 5 inches or less in one season to over 20 in the following. Salinas, in the northern portion of the valley, has an annual rainfall of 13.96 inches; Soledad, in the central part, 8.95 inches; and King City, in the southern portion, 11.32 inches.

The variation in rainfall from year to year in the Salinas Valley has a marked effect on the overwintering adults on the foothills. During successive heavy rainy seasons the spring population of leafhoppers which invaded the cultivated areas was often reduced to a minimum, and average or high yields of beets were often obtained. The seasonal rainfall during 1914-15 was 15.72 inches at the Spreckels ranch near King City and no leafhoppers or curly top were reported to have occurred in the beet fields. During 1909 an average of 15.37 tons per acre were harvested on the Spreckels ranch near King City and the seasonal rainfall was 15.64 inches. On the other hand, during 1907 an average of only 8.6 tons per acre were harvested, yet the seasonal rainfall was 19.98 inches; 12.00 and 13.75 inches had occurred during the two previous rainy seasons.

According to the Weather Bureau of the United States Department of Agriculture the seasonal rainfall at King City was as follows: 1914-15, 11.50; 1908-09, 13.51; 1906-07, 20.54; 1905-06, 12.91; 1904-05, 14.33 inches.

The seasonal rainfall at King City during severe outbreaks of the beet leafhopper is shown in table 4.

TABLE 4  
SEASONAL RAINFALL IN THE KING CITY DISTRICTS DURING  
OUTBREAKS OF BEET LEAFHOPPER

Year	Spreckels ranch near King City	U. S. Department of Agriculture Weather Bureau, King City
	<i>inches</i>	<i>inches</i>
1898-99.....	7.07	7.07
1899-1900.....	8.60	7.42
1900-1901.....	16.40	16.22
1904-05.....	13.75	14.33
1913-14.....	15.64	14.61
1918-19.....	11.09	8.78
1921-22.....	13.15	12.12
1924-25.....	5.86	6.25
1926-27.....	10.29	9.79

During 1899, 1900, and 1901 irrigation facilities when needed were inadequate, but the failure of the sugar-beet crop could largely be attributed to curly top. During 1919, 55 per cent of the sugar beets were infected with curly top before the spring flights occurred from the foothills (fig. 22) by the overwintering adults which had remained in the cultivated areas. The migrations of the leafhoppers from the San Joa-

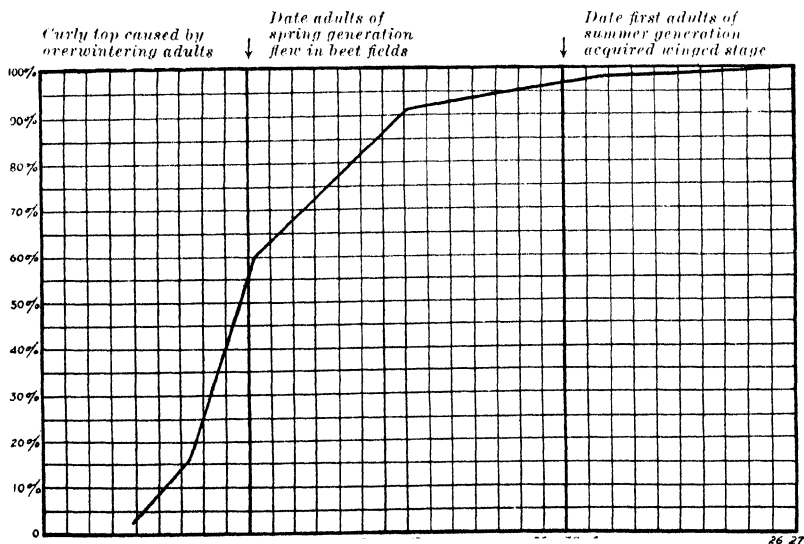


Fig. 22. Chart showing that 55 per cent of the beets in the Salinas Valley were infected with curly top by the dark overwintering adults which occurred in the cultivated areas of the Salinas Valley before the first adults of the spring generation flew into the beet fields. (Adapted from a chart by W. W. Thomas, formerly employed by the Spreckels Sugar Company.)

quin into the Salinas Valley during 1922, 1925, and 1927 have already been discussed. During 1901, 1905, and 1914 the rainfall was sufficient to reduce the population of leafhoppers on the Salinas foothills, and the severe outbreaks of the leafhoppers must have been due to migration from the San Joaquin Valley.

A comparison of the seasonal rainfall from north to south in the San Joaquin Valley with King City in the Salinas Valley is made in table 5.

TABLE 5  
COMPARISON OF RAINFALL FROM NORTH TO SOUTH IN SAN JOAQUIN VALLEY  
WITH KING CITY, SALINAS VALLEY

Station	1900-01	1904-05	1913-14	1921-22	1924-25	1926-27	Average annual rainfall
Tracy.....	14. 10	15. 05	12. 17	.....	.....	10. 51	.....
Westley.....	13. 71	11. 65	17. 08	7. 98	.....	.....	.....
Newman.....	12. 08	14. 42	16. 20	.....	9. 46	10. 55	.....
Los Banos.....	11. 37	11. 83	.....	.....	.....	8. 20	.....
Firebaugh.....	.....	.....	9. 70	10. 38	7. 70	6. 40	.....
Mendota.....	10. 89	9. 53	.....	.....	.....	.....	.....
Coalinga.....	.....	.....	9. 65	9. 62	4. 64	8. 34	7. 24
Dudley.....	.....	.....	8. 14	8. 14	5. 36	7. 67	7. 07
Antelope Valley.....	.....	.....	10. 61	11. 61	4. 62	.....	.....
Bakersfield.....	.....	8. 27	7. 35	8. 88	4. 69	6. 20	5. 68
Maricopa.....	.....	.....	9. 97	8. 75	3. 52	7. 30	6. 36
King City*.....	16. 40	13. 75	15. 64	13. 15	5. 86	10. 29	.....
King City†.....	16. 22	14. 33	14. 61	12. 12	6. 25	9. 79	11. 32

\* Spreckels ranch near King City.

† U. S. Department of Agriculture Weather Bureau, King City.

It is evident from table 5 that during the 1900, 1905, and 1914 outbreaks of the pest the seasonal rainfall was unfavorable in the northern San Joaquin Valley but favorable in the middle and southern parts of the valley.

Dessicating winds in the Salinas Valley may be an important indirect controlling factor affecting the water balance of the leafhopper, especially when it feeds on weeds which wilt and become sunscorched during hot days. During extremely hot spells the leafhoppers will leave wilted, sunscorched weeds and seek other food plants; but the change to certain food plants results in a mortality of the nymphs and adults.

*In Coastal Regions.*—Fog is the most important barrier when the beet leafhoppers migrate into the coastal regions. During the 1925 outbreak, the insects migrated across San Pablo Bay into the beet fields along the coast near Ignacio. The April plantings showed an average of 60 per cent curly top on July 15, while in the June plantings no curly

top or beet leafhoppers were found. A single nymph was found in the April plantings during a half day's search. An examination of the same fields on August 1 showed that all of the beets in the April plantings were diseased, while in the June plantings 16 per cent of the beets adjacent to the April plantings, were diseased and an average of 10 per cent of the beets in isolated June plantings showed curly-top symptoms. No fungus-diseased leafhoppers were found. Most of the offspring of the spring migrants were probably exterminated by fogs, possibly augmented by low temperatures associated with them.

In determining the life history of the leafhopper in the fog belt at Berkeley, 22 adults of the first generation were reared between May 15 and June 23. A single adult of the second generation was bred from eggs deposited by the 22 adults on October 21.<sup>(30)</sup> The egg-laying capacity of a single female kept out of doors at Manteca situated in the northern San Joaquin Valley was 328 eggs.<sup>(35)</sup> Fogs and probably low temperatures were the limiting factors which prevented a large population from developing in cages out-of-doors.

## FLUCTUATIONS IN POPULATION

*Early Drying of Pasture Vegetation.*—The factors associated with the reduction in numbers of the spring generation vary in different years. In some years dessicating northerly winds dried the pasture vegetation rapidly during the spring and hence large numbers of eggs failed to hatch. In all probability when red-stem filaree becomes wilted many eggs fail to hatch, as is the case when sugar-beet leaves and weeds wilt in the greenhouse.

The primary cause for the enormous reduction in numbers of the beet leafhoppers on the uncultivated plains and foothills during 1923 was the early drying of the pasture vegetation. Drought conditions from the middle of February to the close of March dried the filaree in March instead of in April and May as in a normal season. In some years the pasture vegetation dried so rapidly in the southern section of the San Joaquin Valley that many nymphs failed to acquire the winged stage. During the spring of 1931, however, when a low population of leafhoppers occurred in Little and Big Panoche passes owing to the rapid drying of the pasture vegetation during early April, a high population was present in the southern San Joaquin, where more rainfall kept the pasture vegetation green.

*Destruction of Pasture Vegetation by Aphids.*—During the spring of 1927 aphids were extremely abundant in the middle San Joaquin and



destroyed most of the red-stem filaree during March, and thus reduced the food supply before many of the leafhopper nymphs acquired the winged stage. According to Schwing, 90 per cent of the red-stem filaree was destroyed on the foothills in the vicinity of Coalinga. In Little and Big Panoche passes most of the red-stem filaree was also destroyed by aphids.

*Early Autumn Rains.*—One factor favorable for the increase of the beet leafhopper during the 1919 outbreak of the pest was the heavy rainfall on September 11 to 13, 1918, germinating the seeds of the pasture vegetation on the uncultivated plains and foothills, and resulting in a new growth of weeds in the cultivated areas of the San Joaquin and Salinas valleys. During the autumn the saltbushes and other favorable host plants of the leafhopper normally become dry. In 1918, however, the nymphs which hatched from eggs deposited in the fall by the females of the summer generation found an abundance of food in this new growth of vegetation in the cultivated districts. Many of these nymphs acquired the winged stage after the return flights of the overwintering adults to the plains and foothills during October and November.

Nymphs were also taken during November and December, 1918, on red-stem filaree growing on the foothills of the San Joaquin and Salinas valleys. These nymphs hatched from eggs deposited in filaree by the females of the summer generation and not by the overwintering forms.

*Late Spring Rains.*—A partial second brood develops on the uncultivated plains and foothills of the San Joaquin Valley whenever late spring rains occur and the pasture vegetation remains green. As already stated, a partial second brood developed on the foothills of the Salinas Valley during the 1925 outbreak of the pest.

*Spring and Summer Migrations.*—Spring migrations reduce the number of beet leafhoppers, since no return flights to the natural breeding areas occur.

Summer migrations may deplete the natural breeding grounds of the beet leafhopper to a large extent; the nymphs and adults are then hard pressed by parasites and predaceous enemies. The observations on the summer migrations from the San Joaquin Valley during 1919 have been given in this paper.

## NATURAL ENEMIES

## PREDATORS

According to Hartung<sup>(17)</sup> three predaceous bugs prey on the beet leafhopper in California: *Neides muticus* Say; *Zelus socius* Uhl; and *Reduvicolis kalmi* Reut.

Specimens of *Geocoris pallens* Stål were frequently seen in the field sucking out the juices of nymphs and adults. A reddish mite attached to the body of the beet leafhopper was sometimes observed (plate 4, *B*).

During 1925 the green lacewing (*Chrysopa californica* Coq.) (fig. 23) was extremely abundant in the beet fields about 5 miles west of Terminus in the San Joaquin Valley. The eggs (fig. 23 *B*) were found on the blades and petioles of every beet examined. The green-lacewing larva devoured the nymphs and adult leafhoppers in cages.

Spiders were noticed feeding on the leafhoppers on the uncultivated plains and foothills and in the cultivated areas.

In the greenhouse control measures must be adopted against the Argentine ant (*Iridomyrmex humilis* Mayr), which enter the cages, kill the nymphs, and occasionally the adults, and carry them to their nests.

## PARASITES

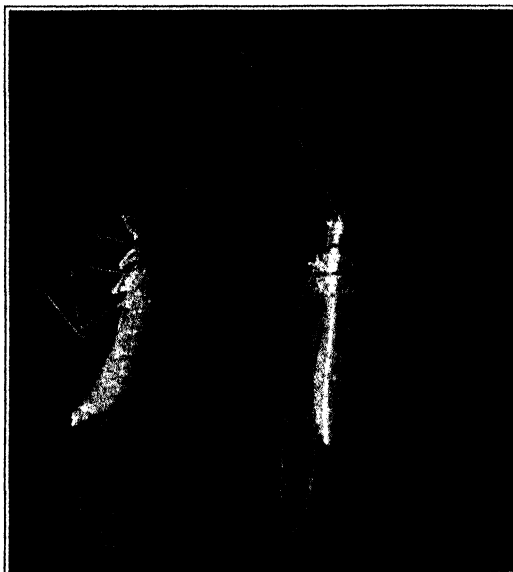
Hartung<sup>(17)</sup> bred three egg parasites from the eggs of the beet leafhopper; these were determined by A. A. Girault to be *Abbella subflava* Gir.; *Anaphes* sp. near *hercules*; and *Gonatocerus* sp. The last two egg parasites emerged from eggs of the leafhopper from Ravendale, Lassen County, California.

Stahl<sup>(41)</sup> bred *Abbella subflava* Gir. from the eggs of the beet leafhopper at Riverside and called attention to the fact that this is a primary parasite and not a hyperparasite as Hartung<sup>(17)</sup> states. Stahl reared two egg parasites, *Polynema eutettigis* Gir. and *Anagrus giraulti* Craw., at Spreckels and Riverside.

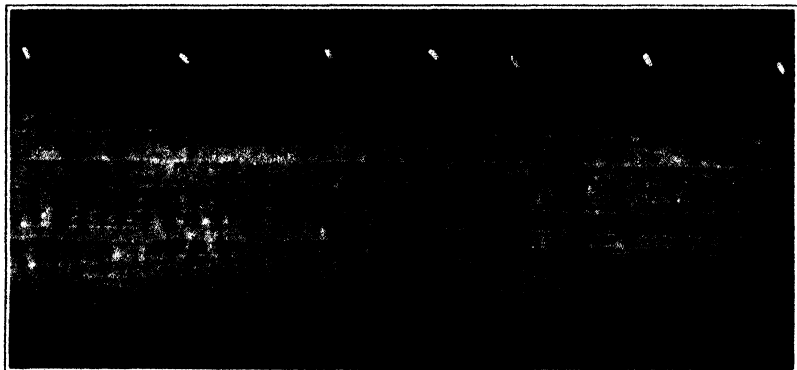
Severin<sup>(31)</sup> bred four egg parasites from the eggs of the beet leafhopper in the San Joaquin Valley: *Polynema eutettigis* Gir. (plate 5, *A, B*); *Anagrus giraulti* Craw. (plate 5, *C, D*); *Apelinoidea plutella* Gir.; and *Anthemiella rex* Gir. These egg parasites were reared more abundantly from eggs deposited by the leafhopper in saltbushes than in sugar beets.

The writer, in cooperation with C. F. Henderson, bred a single specimen of *Ufens* n. sp., presumably from eggs of the beet leafhopper de-

posited in red-stem filaree growing in the Little and Big Panoche passes during the spring of 1928. *Aphelioidea plutella* Gir. has been bred from eggs deposited in red-stem filaree collected in the vicinity of Coalinga,



A



B

Fig. 23. Green lacewing (*Chrysopa californica* Coq.): A, adults;  
B, eggs deposited on the petiole of a sugar-beet leaf.

but no egg parasites other than these two have been reared from material collected on the plains and foothills up to the present time.

Two parasitic flies were bred from the beet leafhopper: *Pipunculus vagabundus* Knab (plate 6, A, B) and *P. industrius* Knab. The *Pipun-*

*culus* flies deposit an egg in the nymph or adult leafhopper. The egg hatches into a larva or maggot which feeds within the abdomen of the leafhopper (plate 6, *D*). When the larva is full grown (plate 6, *E*) it bores out of the host, leaving an exit hole (plate 6, *F*), usually near the junction of the metathorax and abdomen. The leafhopper is killed after the larva emerges. After escaping from its host, the larva buries itself beneath the soil and pupates (plate 6, *G*). At Spreckels the flies issued after remaining in the pupal stage (plate 6, *H*) for a period of 22 days during the summer.<sup>(19)</sup>

A wingless female ant-like wasp, *Gonatopus contortulus* Patton (plate 4, *A*; plate 7, *B*), and the winged male (plate 7, *A*) were bred from the beet leafhopper. The ant-like female is a very active creature, capturing and partly devouring a large number of leafhoppers. A single parasite emerging in a cage will kill most of the leafhoppers, but only in an occasional host is an egg deposited. The presence of the parasite in its later development can be determined by the external brown sac in the nymph or beneath one of the wings in the adult leafhopper (plate 7, *C*). After the full-grown larva bores out of the leafhoppers (plate 7, *D*, *E*) it leaves in the exit hole the larval sac consisting of the molted skins of the larva (plate 7, *F*). The larva spins a white cocoon (plate 7, *H*) 3 mm long and 1 mm wide, on the foliage of the saltbush or beet leaf. Forty days after spinning its cocoon the wingless parasite emerged on October 24, 1914, at Spreckels.<sup>(19)</sup>

In 1913 about 3.2 per cent and in 1914 about 33.6 per cent of the beet leafhoppers collected in the beet fields were found to be parasitized by *Pipunculus vagabundus*, *P. industrius*, and *Gonatopus contortulus*.<sup>(19)</sup>

During 1918-1920 a comparison was made of the percentage of parasitized beet leafhoppers collected on the uncultivated plains and foothills with those captured in the cultivated areas of the San Joaquin Valley. Records obtained by dissecting the adult were more reliable than those secured by breeding the parasites, since a high mortality of the insects occurred in the breeding jars. The average percentage of parasitized adults of various generations by *Pipunculus* and *Gonatopus* is indicated in table 6.

According to table 6, in 1919 the percentage of parasitism gradually increased during the summer months in the cultivated areas and reached its height during August. The weak point in the parasitism occurs on the uncultivated plains and foothills. Table 6 shows that 4.4 per cent of the overwintering adults collected on the plains and foothills were parasitized compared with 28.0 per cent of the overwintering adults captured in the cultivated areas. Dissections show that leafhoppers of the

autumn generation parasitized by a large larva remain in the cultivated areas, although leafhoppers parasitized by a tiny larva fly to the foothills.

A parasitic hairworm (plate 4, *C, D*) belonging to the Gordiaceae emerged and was also dissected on rare occasions from the beet leafhopper. A case of double parasitism by a hairworm and a *Pipunculus* larva occurred in the abdomen of an overwintering female collected on the foothills near King City in the Salinas Valley on November 28, 1918.

TABLE 6

PARASITISM OF BEET LEAFHOPPER ON PLAINS AND FOOTHILLS AND IN CULTIVATED AREAS OF SAN JOAQUIN VALLEY

Uncultivated plains and foothills				Cultivated areas			
Year	Months	Generation	Average percentage parasitism	Year	Months	Generation	Average percentage parasitism
1918-19	Nov.-Mar.	winter	8.2	1918	Dec.	winter	28.0
1919	Apr.	spring	1.0	1919	Apr.-May	spring	3.5
1919-20	Oct.-Feb.	winter	4.4		June	summer	10.0
1920	Apr.	spring	1.5		July	summer	22.3
					Aug.	summer	35.1
					Sept.	summer	32.0
				Average, June-Sept.	summer	24.8	

The beet leafhopper is also parasitized by an occasional *Stylops*, which was not bred.

Since it had been reported by Bonequet<sup>(4)</sup> that the beet leafhopper, as well as curly top of sugar beets, occurred in Argentina, an exploration for parasites of this insect was undertaken in that country by the University of California in cooperation with the United States Department of Agriculture, Bureau of Entomology.

According to Bonequet<sup>(4)</sup> the beet leafhopper has been found in the vicinity of "Buenos Aires, in Guatrache, Alpachiri, and Bahia Blanca (southeast), in Colonia Alvear and Mendoza (west) covering the major part of the temperate and subtropical zones of the Argentine Republic." Leafhoppers which were reported to have been collected at San Isidro, a suburb of Buenos Aires, were determined as *Eutettix tenellus* (Baker) by E. P. Van Duzee and the writer, and are in the collections of the California Academy of Sciences.

Henderson, who was sent to Argentina to introduce parasites of the beet leafhopper into the United States, swept the most favorable host plants of the beet leafhopper with an insect net in the localities reported

by Bonequet, and many other localities, from November 11, 1926, to June 30, 1927, but failed to find *Eutettix tenellus*. Insect collections in museums and of entomologists were examined, but not a single specimen of *E. tenellus* was found. An undetermined species of *Eutettix* resembling the general shape and size of *E. tenellus* was collected by Henderson on sugar beets, garden or red beets, and Swiss chard in every locality in which Bonequet reported that *E. tenellus* occurred except in Guatrache.<sup>(36)</sup> Henderson<sup>(20)</sup> has published a detailed report on the exploration in Argentina for the beet leafhopper.

Fawcett<sup>(13, 14)</sup> demonstrated that a disease of sugar beets in Argentina resembling the foliage symptoms of curly top in North America, was transmitted by *Agallia sticticollis* Stål.

Since the beet leafhopper was known to occur in the western part of North America from Canada into Mexico, an exploration of Mexico for parasites of the leafhopper was undertaken, for it was thought that possibly the original native home of the insect was Mexico, and that the insect through migratory flights had established itself in localities outside the range of its efficient parasites.

Henderson<sup>(7)</sup> explored Lower California, the west coast and the central district of Mexico, Arizona, Utah, and southern Idaho for parasites of the beet leafhopper during the period from October 3, 1927, to July 16, 1928. He states: "Neither the beet leafhopper nor parasites were found on the high central plateau, which extends from the southern portion of Durango to Mexico City. For the west coast, although the territory from Nogales, Arizona, to Mazatlan, Sinaloa, was covered, the range of the leafhopper apparently extended only as far south as Guasave, Sinaloa." Egg parasites occurred over the entire area occupied by the insect, most of which had previously been bred by other workers from the eggs of the beet leafhopper in California.

#### FUNGUS DISEASE

Fungus diseases kill some of the overwintering leafhoppers and spring migrants (plate 8), as reported in previous papers.<sup>(28, 31)</sup> During December, 1918, large numbers of leafhoppers were collected on the foothills bounding a canyon in the northern San Joaquin Valley 13 miles southwest of Tracy. When these insects were confined in cages in the greenhouse at Berkeley, they died as a result of a fungus disease. The weather records kept by the Spreckels Sugar Company at Manteca showed that the precipitation from September to April was 17.29 inches; 9.98 inches of rain fell from September to December. During the

1919 outbreak of the pest, the spring migrants succumbed to a fungus disease in the fog belt of San Luis Obispo and Santa Barbara counties. An examination of the lower surface of the leaves of a single sugar beet showed 178 jassids, including beet leafhoppers, which had died as a result of a fungus disease. In regions outside of the fog belt, however, no dead fungus-diseased insects were found that year, and near Los Alamos, nymphs and adults were abundant in the beet fields.

### SUMMARY

The northern limit of the breeding range of the beet leafhopper in the San Joaquin Valley was found to be in a canyon in the Mount Diablo Mountains situated about 4 miles southwest of Pittsburg. The natural breeding area includes the canyons of the Mount Diablo Range in northern section, the plains and foothills of the Inner Coast Range in the middle and southern sections, and the foothills of the Tehachapi Mountains in the southern section of the San Joaquin Valley. The plains and foothills of most of Kern County are natural breeding grounds, except the Sierra Nevada foothills near the northern end of the county. The northern limit of the breeding range on the Sierra Nevada foothills was found to be about 10 miles north of Porterville near Lindsay in Round Valley.

A natural breeding area occurs between the Coast Ranges on the western foothills of the Panoche Hills bounding Panoche Valley.

The northern limit of the foothill breeding range on the Gabilan Mountains in the Salinas Valley is at the boundary of the fog belt south of Soledad and the southern limit in the vicinity of San Miguel, the most favorable foothill breeding area being from Greenfield to Bradley.

A natural breeding area extends from Santa Ana Valley to Panoche Pass, becoming less favorable toward Pacheco Pass.

A natural breeding grounds occurs in Honey Lake Valley at an altitude of about 4,000 feet in the Sierra Nevada. The beet leafhopper was also found in the Sierra Valley and was reported to occur in the American and Indian valleys.

The beet leafhopper was taken on twenty species of food plants growing on the uncultivated plains and foothills, five of which belong to the Chenopodiaceae, to which the sugar beet belongs. The nymphs were bred from eggs deposited in eight different species of plants under natural conditions. Red-stem filaree is the most important host plant upon which the overwintering adults feed and deposit their eggs, and upon which the spring generation develops.

When the beet leafhopper is abundant it occurs on most weeds and a large number of economic plants. Nymphs have been bred from eggs deposited in forty-six different annual and perennial plants growing in the cultivated areas; nineteen of these breeding plants belong to the *Chenopodiaceae* and the remainder to twelve other families.

The range of the beet leafhopper corresponds to the geographical distribution of the saltbushes. Further breeding experiments are necessary to determine whether the native mustards were the original host plants of the leafhopper.

The spring dispersal of the leafhopper from the uncultivated plains and foothills occurs after the pasture vegetation becomes dry and is probably associated with a food stimulus, the insects invading the cultivated areas when the annual saltbushes and other weeds are succulent and most favorable from the standpoint of food and egg deposition.

The spring flights have frequently been observed from the entrance of Little Panoche Pass and appear to be associated with air currents at sunset. The ascending air currents may carry the insects into the higher winds and here they may drift and fly long distances, possibly over mountain ranges. When the pest was at the maximum in numbers immense swarms flew into the cultivated areas. One swarm from the plains and foothills of the middle San Joaquin flew across the valley, a distance of about 50 miles. A succession of northward flights occur in the cultivated areas of the San Joaquin Valley and apparently the insects fly against light northwest winds.

A spring migration of the leafhopper occurs from the cultivated areas of the San Joaquin into the Sacramento Valley. Flights of small numbers of leafhoppers precede the large migration into the Sacramento Valley. Spring migrations from the San Joaquin Valley have occurred across Suisun and San Pablo bays, into Livermore Valley, San Francisco Bay districts, Santa Clara, San Juan and Salinas valleys, and possibly into the fog belt of the southern counties. The distance of a migratory flight from the San Joaquin into the Sacramento Valley was estimated at about 60 miles, and successive northward migrations following the cultivated areas of the Sacramento Valley at about 150 miles.

The dispersal of the summer generation from badly diseased beets to healthy beets is known to extend at least 3 miles in the Sacramento Valley.

Summer migrations of the beet leafhopper from the San Joaquin Valley occurred during the 1919 outbreak of the pest. These migrations were probably associated with overcrowding and unfavorable food.



The autumn dispersal from the cultivated areas to the uncultivated plains and foothills in the San Joaquin and Salinas valleys occurs during October, November, and December. During the autumn dissemination the insects congregate on favorable weeds growing on abandoned farms on or near the plains. Frequently the lines of flight across the plains to the canyons and mountain passes follow dry creek beds where the insects take short flights from perennial to perennial. The leafhoppers also occur on perennials growing on the plains. The autumn flights of the leafhopper are probably associated with a food stimulus; the insects fly from the cultivated districts when the saltbushes and other favorable weeds become woody and dry.

The most important natural barrier of the beet leafhopper is rainfall, which reduces the population on the northern foothills of the San Joaquin Valley. The abundance of rainfall in the Sacramento Valley is the factor that exterminates the overwintering adults on the foothills and in the cultivated areas. Rainfall when above normal reduces the population on the foothills in the Salinas Valley. Fog and possibly low temperatures are limiting factors to the offspring of the migrants when the leafhopper migrates into the coastal regions. Various composite controlling factors, such as high humidity owing to rains and fogs, heavy dew, soil moisture, tall dense flaree, cloudiness or low temperatures, may play important roles in the survival of the insect during the hatching and molting process. The succession of favorable food plants throughout the season may also be a limiting factor in certain migratory areas of the insect, such as the middle and northern Sacramento Valley.

The factors associated with the reduction in numbers of the spring generation vary in different years. In some years dessicating northerly winds dry the pasture vegetation rapidly during the spring and hence large numbers of eggs fail to hatch. The primary cause for the enormous reduction in numbers of the spring generation during 1923 was the early drying of the pasture vegetation during March instead of April and May, so that many nymphs died before they acquired the winged stage. During the spring of 1927 aphids destroyed most of the flaree in the middle San Joaquin Valley, reducing the spring population of beet leafhoppers.

The primary cause for the enormous increase of the beet leafhoppers during 1919 hinges on two factors: (1) There were no summer migrations of the pest from the natural breeding grounds during 1918, so that large numbers of eggs were deposited during the autumn; (2) the nymphs which hatched from these eggs found an abundance of green food, not only in the cultivated areas but also on the uncultivated plains

and foothills, after the heavy September rains germinated the seeds of the vegetation.

Another factor favorable for the increase of the beet leafhopper on the uncultivated plains and foothills is late spring rains, which keep the pasture vegetation green so that a partial second brood develops.

Among the natural enemies of the beet leafhopper are a large number of predacious insects which prey upon the nymphs and adults. Seven species of egg parasites, two species of *Pipunculus* flies, a *Gonatopus*, and a hairworm were bred by various entomologists in California. In 1919 the percentage of parasitized leafhoppers gradually increased during the summer months and reached its height during August (35.1 per cent). The weak point in the parasitism of the leafhoppers occurred on the uncultivated plains and foothills where only 4.4 to 8.2 per cent were parasitized during the winter and 1.0 to 1.5 per cent during the spring. No information is at hand as to the value of egg parasites on the uncultivated plains and foothills and in the cultivated areas.

Fungus diseases reduce the numbers of overwintering leafhoppers and spring migrants in the fog belt in favorable years.

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## PLATES



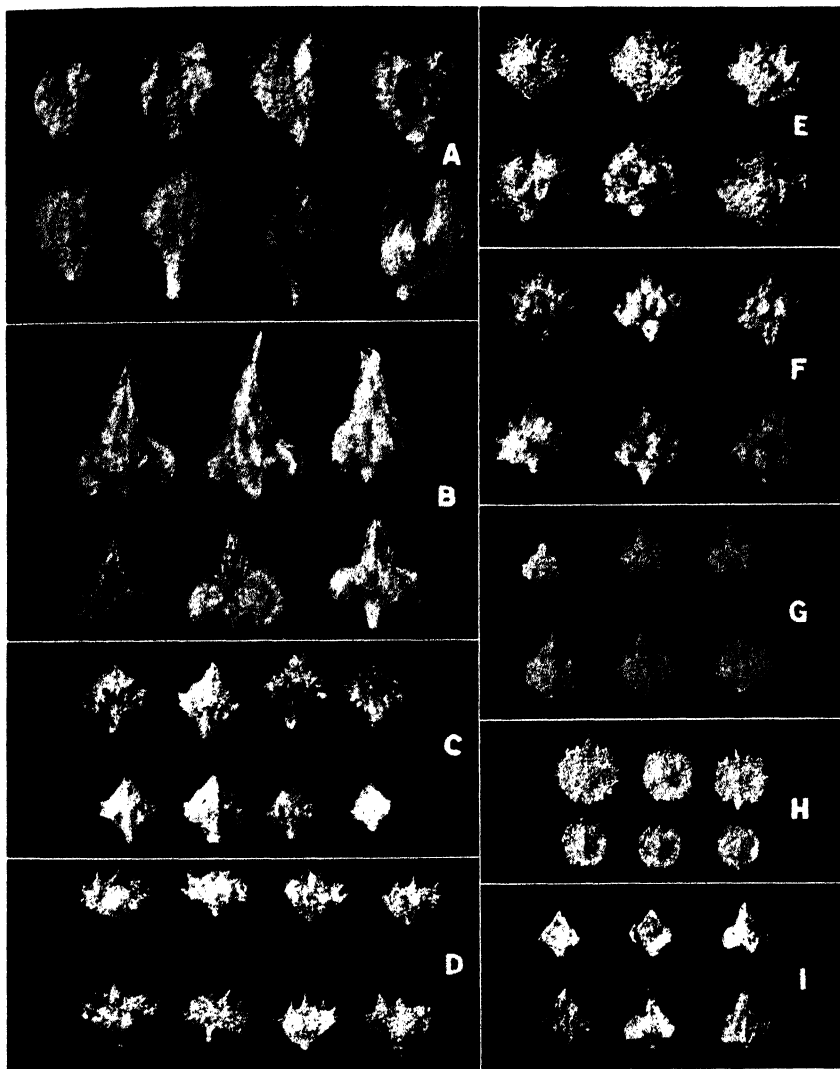


Plate 1. Fruiting bracts of annual saltbushes. These structures have been almost universally considered as modified upper leaves enclosing the seeds. A, fogweed or silver scale (*Atriplex argentea expansa*); B, arrow scale (*Atriplex phyllostegia*); C, red orache, or red scale (*Atriplex rosea*); D, bract scale (*Atriplex bracteosa*); E, crown scale (*Atriplex coronata*); F, heart scale (*Atriplex cordulata*); G, *Atriplex tularensis*; H, wheel scale (*Atriplex elegans*); I, brittle scale (*Atriplex parishi*).



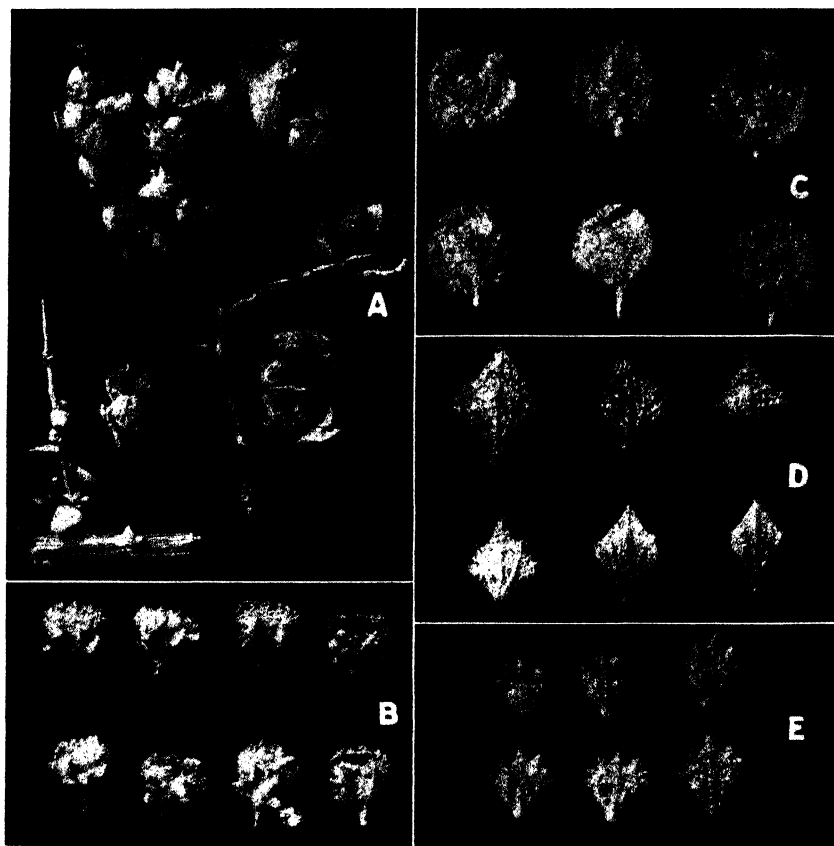


Plate 2. Fruiting bracts of perennial saltbushes: *A*, spinescale (*Atriplex spinifera*), showing clusters of fruiting bracts; *B*, cattle quailbrush or lenscale (*Atriplex lentiformis*), showing clusters of fruiting bracts; *C*, quailbrush or lenscale (*Atriplex lentiformis*), showing compressed rounded fruiting bracts; *D*, fleshscale or Australian saltbush (*Atriplex semibaccata*). Fruiting bracts are convex, fleshy-thickened, and turn red in living plants, but are compressed and nearly flat when dry. *E*, Ballscale (*Atriplex fruticulosa*).

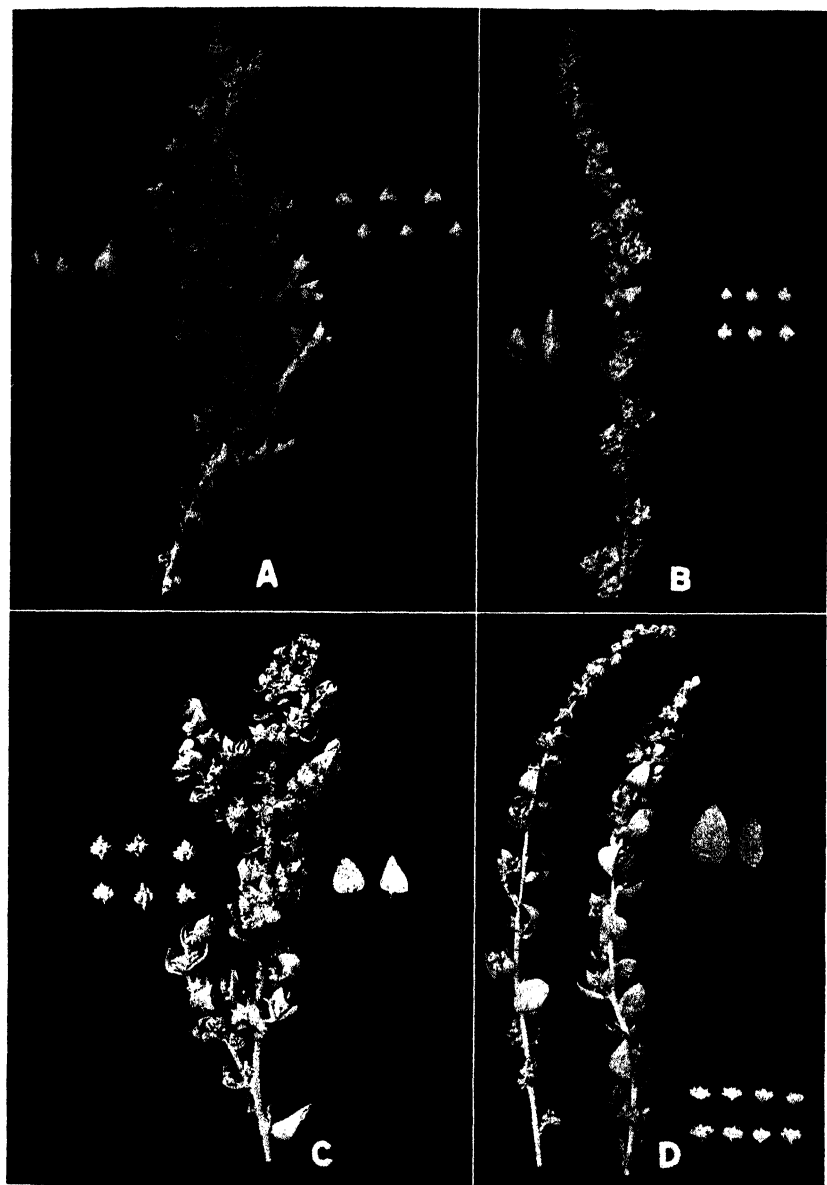


Plate 3. Branches of four annual saltbushes showing leaves and fruiting bracts, also shape of leaves and fruiting bracts removed from plants: A, brittle scale (*Atriplex parishi*); B, *Atriplex tularensis*; C, heart scale (*Atriplex cordulata*); D, bract scale (*Atriplex bracteosa*).

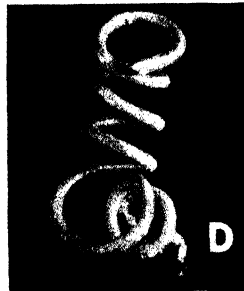
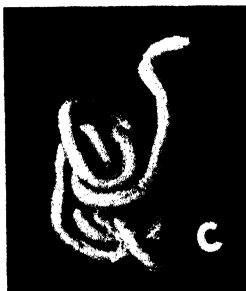
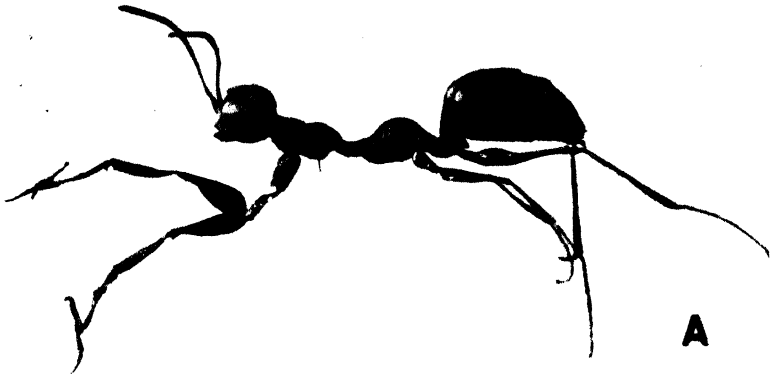


Plate 4. A, Female of *Gonatopus contortulus* Patton showing front legs adapted for grasping prey. B, Reddish mite removed from leg of a beet leafhopper. C, D, Parasitic hairworms of beet leafhopper.

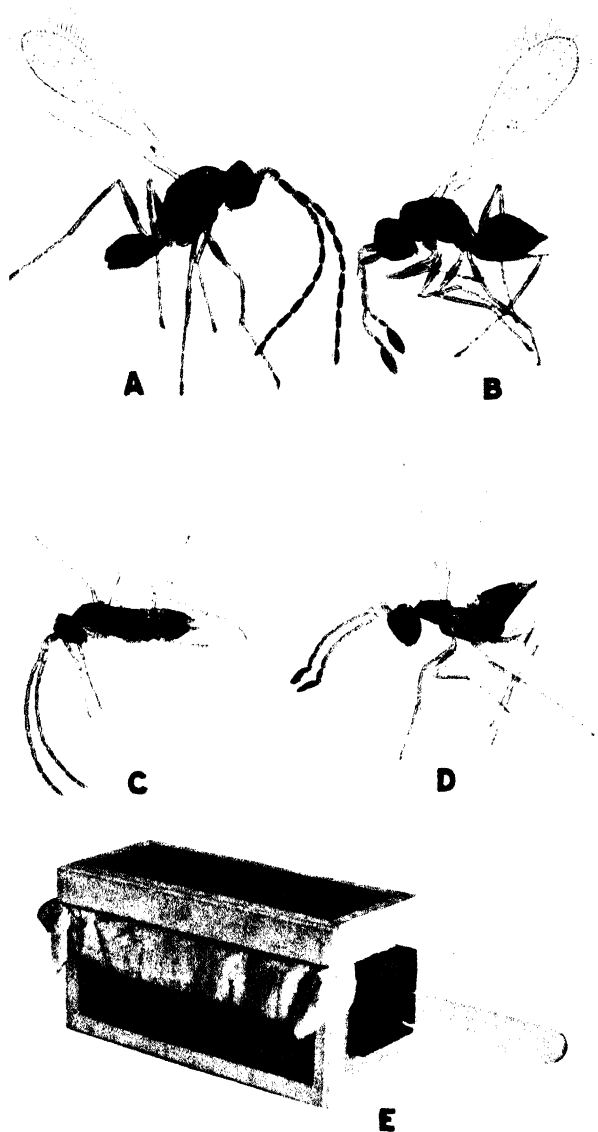


Plate 5. Egg parasites of beet leafhopper and breeding box: A, male of *Polynema cutettigis* Gir.; B, female of *Polynema cutettigis*; C, male of *Anagrus giraulti* Craw.; D, female of *Anagrus giraulti*; E, egg parasite breeding box. The egg parasites upon emerging are positive to light and enter the test tube.

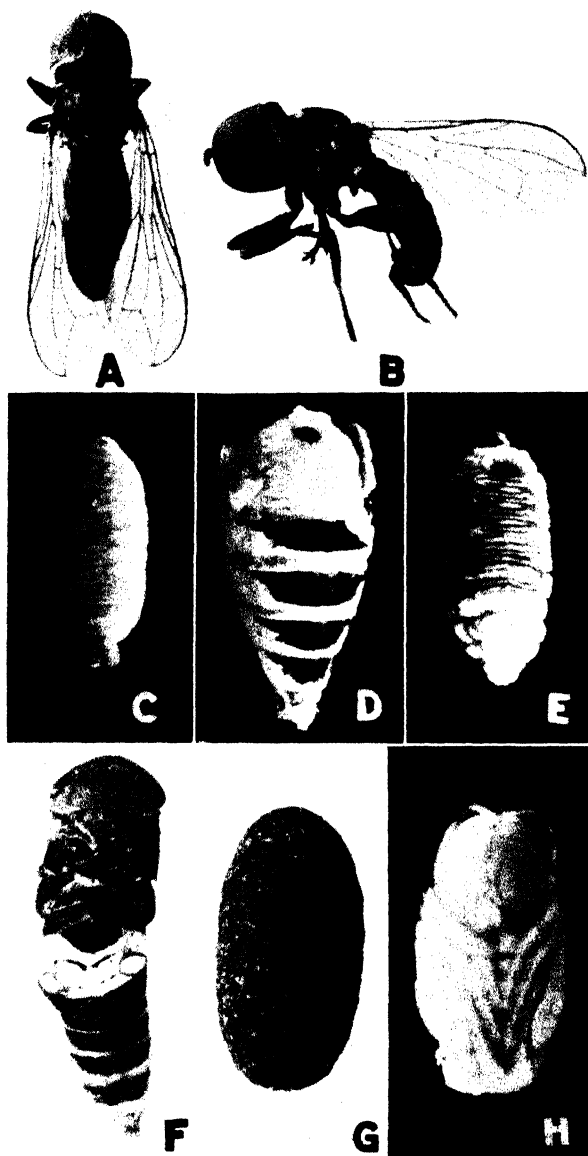


Plate 6. Stages of big headed fly or *Pipunculus*, a parasite of the nymphs and adults of the beet leafhopper: A, male of *Pipunculus vagabundus* Knab.; B, female of *Pipunculus vagabundus*; C, immature larva showing respiratory tubes; D, larva in the abdomen of the beet leafhopper; E, full-grown larva after boring out of the beet leafhopper; F, beet leafhopper showing large exit hole through which a *Pipunculus* larva bored out; G, puparium; H, pupa.

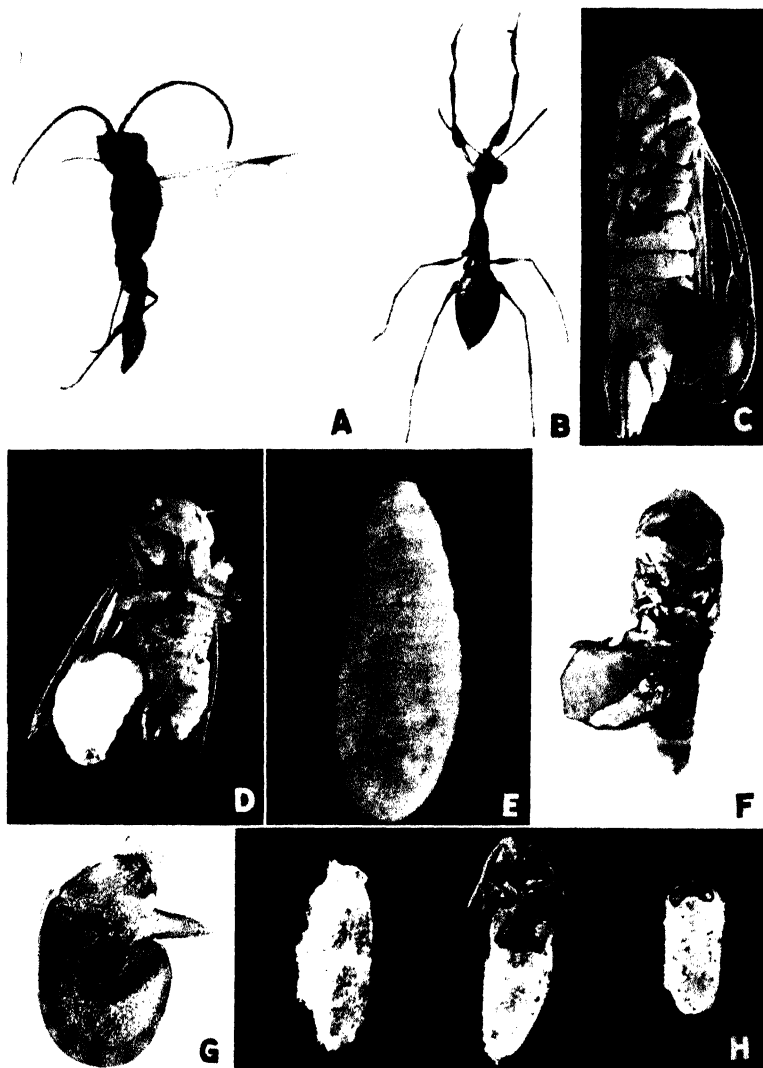


Plate 7. Stages of *Gonatopus contortulus* Patton, a parasite of the nymphs and adults of the beet leafhopper: A, male; B, female; C, dark brown larval sac beneath the wing of the beet leafhopper; D, larva boring out of the beet leafhopper; E, full-grown larva after boring out of the beet leafhopper; F, larval sac consisting of molted skins of larva; G, third instar showing head lobe and ventral larval process; H, left, oval white cocoon; center, female that died in cocoon; right, cocoon showing irregular hole which adult gnawed before issuing.

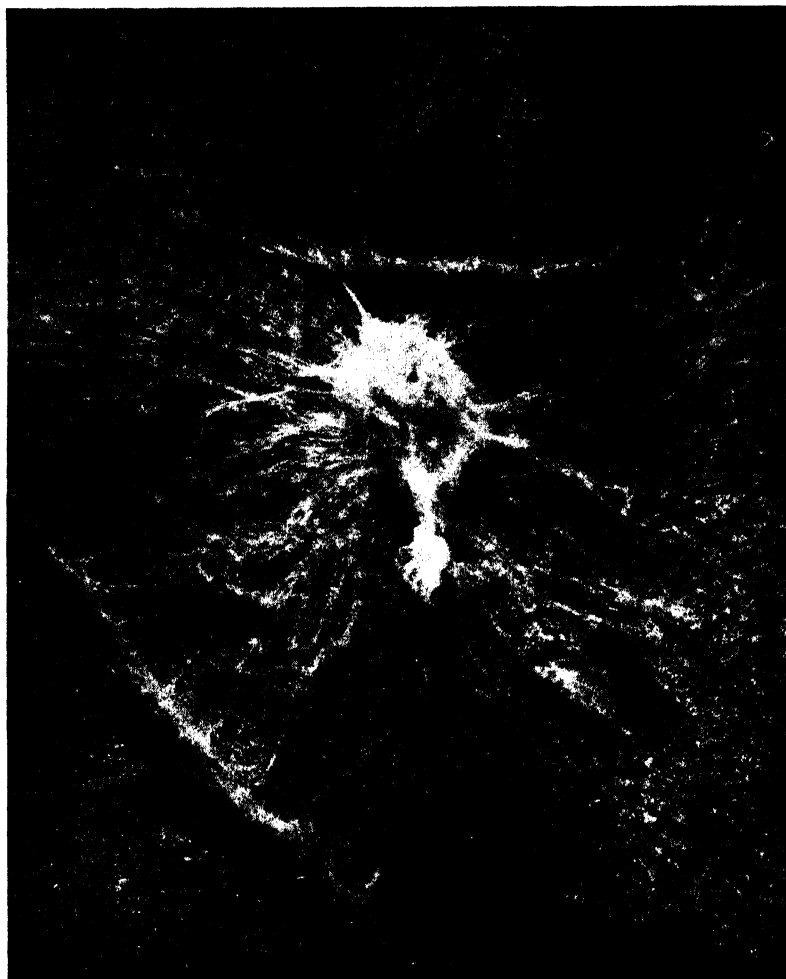


Plate 8. Fungus-diseased beet leafhopper, enlarged, showing mycelium and scattered spores.

# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

APRIL, 1933

No. 9

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## THE USE OF ARSENICAL COMPOUNDS IN THE CONTROL OF DEEP-ROOTED PERENNIAL WEEDS<sup>1</sup>

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### INTRODUCTION

As chemical weed control becomes more generally practiced, the limitations as well as the possibilities of the various methods become more evident. The effectiveness of these methods depends upon certain factors which must be carefully considered if consistent results are to be obtained. Many of the factors may be controlled by the operator. It is, therefore, essential to the successful practice of any new method that a descriptive study of the more readily controlled factors be made; for only when we understand the underlying principles may we obtain the best results.

This preliminary report describes the preparation and use of an arsenical solution recently employed with considerable success in controlling deep-rooted perennial weeds. It discusses the mechanism involved in the movement of the arsenical solution into the roots of plants, and submits data for comparing and evaluating the factors that tend to limit its action. It discusses also the conditions essential to successful practice and gives certain precautions for the handling of the reagents.

One should understand at the outset that the arsenical herbicide described in the following pages will not completely eradicate perennial weeds. Such results cannot be expected of any chemical weed killer. This herbicide has, however, proved very satisfactory when prepared properly and applied under optimum conditions.

<sup>1</sup> Received for publication November 30, 1932.

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Arsenicals have long been utilized in the destruction of undesirable vegetation. Sodium arsenite is the active ingredient in many proprietary herbicides. Although other chemicals have recently been widely used against the more noxious perennial weeds, certain arsenic compounds are cheaper and may be more effective.

In 1917 Gray<sup>(2)</sup> reported that, under some conditions, sodium arsenite solution, applied to the foliage of morning-glory plants, was absorbed and carried into the roots. In 1927 Kennedy and Crafts<sup>(4)</sup> suggested a mechanism which they thought to be responsible for this movement of arsenic compounds within the plant. In 1930<sup>(1)</sup> they gave additional data on their work, citing evidence to confirm their theory. Morgan<sup>(5)</sup> has recently proposed the same mechanism to explain the translocation of arsenic acid in his experiments with hoary cress.

The work presented here further strengthens the theory; and, though the limitations of the method are definitely described, the possibility of developing an efficient control practice seems nearer realization than was apparent in these earlier reports.

The mechanism responsible for the rapid translocation of arsenic compounds into the roots of morning-glory after application to the leaves may be briefly described as follows. When the plant has been transpiring freely for some time, water loss from the leaves exceeds intake by the roots. As the pressure within the xylem becomes sub-atmospheric, a water deficit develops in all living cells. When a plant in this condition is sprayed with a solution of a strong acid or base, the living cells of the leaves and stems are killed and rendered permeable. The pressure gradient becomes immediately effective and all moisture free to move is forced into the xylem and carried down into the roots. If soluble arsenic compounds are present in the spray solution, they will diffuse into the tissues, will become mixed with the vacuolar sap, and, as the cells collapse, will be carried downward along with any unevaporated spray solution. When destruction of the foliage is complete, the tissues become dry, the downward movement in the xylem stops, and the arsenic compounds slowly diffuse from the vessels, killing all living cells of the root. The various factors limiting the action of this mechanism have been described elsewhere in detail<sup>(4, 1)</sup>.

## EXPERIMENTAL RESULTS

Little has been published on the effective concentrations of arsenic compounds and of acids in herbicidal solutions. Gray<sup>(2)</sup> used approximately a 0.5 per cent solution of  $\text{As}_2\text{O}_3$ , and a previous publication<sup>(1)</sup> mentioned  $\frac{\text{M}}{20}$  (approximately 1 per cent) as the optimum concentration.

Tables 1, 2, and 3 present experimental evidence relative to the critical concentrations of these reagents. The plots used were all one square rod in area and were densely infested with morning-glory (*Convolvulus arvensis* L.).

TABLE 1  
EFFECTS OF CONCENTRATION EXPRESSED AS  
PER CENT  $\text{As}_2\text{O}_3$  ON RESULTS OF  
SPRAYING MORNING-GLORY  
WITH ACID ARSENICAL  
SPRAYS

Per cent $\text{As}_2\text{O}_3$	Number of plots	Average per- centage resprouts two months after spraying
4.00	6	9.1
2.00	18	7.2
1.00	18	8.2
0.50	12	8.4
0.25	12	15.8

It is apparent from the data in table 1 that 0.5 per cent  $\text{As}_2\text{O}_3$  is the lowest effective arsenic concentration under the conditions of the experiment and may be considered a lower limit for sprays in field practice.

TABLE 2  
RESULTS OF SPRAYING WITH ARSENICAL  
SOLUTIONS DIFFERING IN ACID  
CONCENTRATION

Normality of acid, $\text{H}_2\text{SO}_4$	Number of plots	Average per- centage resprouts two months after spraying
0.50	25	49.0
1.00	25	9.9
1.50	16	6.1
2.00	25	11.1

As indicated in previous publications,<sup>(1, 3)</sup> a spray of acid reaction is more effective than an alkaline one. The data in table 2 show the relation between acid concentration and effectiveness of the spray.

These figures (table 2) may be compared with some laboratory data (table 3) on the time required for killing morning-glory tissues with acid solutions of differing concentrations. The times of killing were found by allowing leaf sections to stand in acid solutions of determined pH values until death occurred. The pH values in the table were taken from a titration curve on ground morning-glory tissue.

TABLE 3  
TIME REQUIRED FOR KILLING MORNING-  
GLORY TISSUE WITH ACID OF  
DIFFERENT NORMALITIES

Normality of acid $H_2SO_4$	pH attained	Time required for killing, hours
0.41	4.00	48.0
0.50	3.70	16.7
0.75	3.00	4.8
1.00	2.40	1.2
1.25	2.00	0.8
1.50	1.90	0.7
1.75	1.85	0.6
2.00	1.80	0.6

Tables 2 and 3 indicate that the use of a solution sufficiently high in acidity to cause rapid killing of the sprayed foliage is essential in this method of applying arsenicals as herbicides. They further indicate that the acid concentration should be at least 1.0 N to give satisfactory results in the field.

Because the volume of solution available for translocation into the roots is definitely limited by the amount of evaporation of the spray solution occurring after it is applied, the time element in the killing process is necessarily of prime importance. Evaporation being conditioned by temperature and humidity, which are both subject to diurnal variation, certain hours should be best for applying these sprays. Commercial operators have long favored night spraying, the benefits of which have been demonstrated time and again in the field. The data in table 4 offer further substantiation. They are not given as complete proof, but merely to show the magnitude of differences to be expected.

Although the factors affecting evaporation also condition transpiration and thus the water deficit in the plant, water is absorbed only slowly by the roots, especially from soils that are low in moisture; and the deficit remains high for several hours after sundown.

TABLE 4  
INFLUENCE OF TIME OF DAY UPON RESULTS OF SPRAYING  
WITH ACID ARSENICAL \*

Plot No.	Date of application	Time of application	Percentage resprouts two months after spraying
1	Sept. 8	8:00 p.m.	5
2	Sept. 9	8:30 a.m.	20
3	Sept. 9	11:00 a.m.	15
4	Sept. 9	1:30 p.m.	10
5	Sept. 9	4:00 p.m.	10
6	Sept. 9	6:30 p.m.	5
7	Sept. 9	9:00 p.m.	5

\* Solution containing  $\text{H}_2\text{SO}_4$  of 1.0 N concentration and 1 per cent  $\text{As}_2\text{O}_3$ .

Plant physiologists generally agree that considerable energy is used in absorbing moisture from soils, especially when the moisture content approaches the permanent wilting percentage. This competition between the soil and the plant for water is reflected in the water content of the plant and also in the effectiveness of acid arsenical sprays as related to tension in the xylem vessels. The results of the following experiment (table 5) indicate that distribution of arsenical compounds is not effective in plants too abundantly supplied with moisture. Eight plots, located in an orchard, were used. Four had been recently irrigated, so that the soil was still very damp. The plants were making rapid growth and had formed a dense mat of foliage. The other four plots had not been irrigated for six weeks. Though the plants had an abundance of foliage, end growth had practically ceased, and their appearance evidenced the lack of available moisture.

Here again (table 5) the data are given as a confirmation of results observed many times in the field. As the author has already pointed out, <sup>(1)</sup> Gray's recommendation of fall spraying has probably more bearing on soil moisture conditions than on movement of organic foods in the plant. The figures in table 5 indicate that little is accomplished by spraying plants which are growing in a soil saturated with moisture. If top growth is cut off until the soil becomes drier, however, too little foliage may be subsequently produced, and poor results will follow. One should, apparently, allow a maximum growth of tops to develop and then depend upon this large leaf surface to deplete the soil moisture until a high deficit is produced. Only where an ample amount of foliage exists is this type of treatment satisfactory.

The spray solution applied to the plots mentioned above (table 5) was low in acidity (approximately 0.5 N), a condition which, because

of its effect upon penetration, tended to emphasize the differences between treatments on wet and dry soils. The effects of diurnal variation in evaporation rate upon distribution of the arsenical solution are also shown.

TABLE 5  
INFLUENCE OF SOIL MOISTURE ON RESULTS OF SPRAYING WITH  
ACID ARSENICALS

Soil dry			Soil moist, recently irrigated		
Plot No.	Time of application	Percentage resprouts two months after spraying	Plot No.	Time of application	Percentage resprouts two months after spraying
1	2:00 p.m.	75	5	2:30 p.m.	100
2	3:00 p.m.	50	6	3:30 p.m.	100
3	4:00 p.m.	10	7	4:30 p.m.	100
4	7:30 p.m.	5	8	8:00 p.m.	75

When the foliage of sprayed plants has become completely killed and all free moisture has moved down into the roots, rapid mass flow of the arsenical solution ceases; the effectiveness of the application is fixed by the extent of distribution at this time. If more moisture is applied to the tops of the plants before the water columns of the xylem become disrupted, downward flow should be resumed, and distribution of the arsenic compounds should become more extensive. Plots 3 and 7 in table 5 were sprayed with several light applications of water soon after treatment with the chemical solution and plots 4 and 8 were sprayed with water early on the following morning. This procedure, in addition to the favorable time of application, probably explains the excellent results obtained on the dry plots with this particular spray solution.

TABLE 6  
THE EFFECTS OF ADDED APPLICATIONS OF WATER UPON THE RESULTS  
OF APPLYING ACID ARSENICALS

Number of applications of water	Series 1		Series 2		Series 3	
	Number of plots	Average percentage resprouts two months after spraying	Number of plots	Average percentage resprouts two months after spraying	Number of plots	Average percentage resprouts two months after spraying
0	6	19.1	12	6.3	12	14.6
1	6	8.3	....	.....	....	.....
2	6	5.0	....	.....	....	.....
3	....	.....	12	3.0	12	11.6

The results of the practice are further illustrated in table 6. Three series of plots received the acid arsenical spray in the evening. In the first series 6 plots were sprayed twice with water on the following morning, 6 plots received one application, and 6 plots were untreated. In the second and third series, 12 plots in each received three applications on the following morning, while 12 received no water.

Although these three series of plots differed considerably in their general results, because of differences in time and manner of application, all showed consistently less resprouting where water was applied to the dead tops on the morning after the original spraying. Application of water seems, therefore, to be a cheap and easy method of increasing the effectiveness of spraying with acid arsenicals.

## DISCUSSION

Though the data presented on some phases of this work are not extensive, and many experiments are still in progress, a few essential points seem well established. A simple, workable method for preparing an acid arsenical solution in the field is described in the next section. Experiments now going on indicate that little can be hoped for in the improvement of this basic formula, at least until the chemistry of the solutions has been more thoroughly studied.

The field trials emphasize the importance of temperature, incident radiation, humidity, and air velocity on evaporation, and consequently upon the action of the mechanism involved. Once a given solution has been applied to the foliage of the plants, the volume of liquid that carries arsenical compounds into the roots depends directly upon the rate of penetration and translocation and is inversely proportional to the amount of evaporation occurring. When the application is made before sundown during the summer in the central valleys of California, the temperature is usually high, the incident energy intense, and the relative humidity low. Though the high temperature causes rapid penetration, all three of these factors favor a high rate of evaporation. Plot tests and field trials show that at this time of day insufficient solution reaches the roots to provide for a thorough distribution.

After dark the temperature drops, radiation is practically eliminated, and relative humidity goes up. Evaporation, in consequence, is very much reduced; and though penetration is less rapid, the acidity of the solution can be adjusted to keep it optimum. The total volume of liquid, including the vacuolar sap of the plant cells, which is available for translocation, is greater. The xylem tissues of the root system therefore

become completely filled with arsenical solution, and injury is much more general. Strong winds at any time increase evaporation rate and lower the effectiveness of the treatment.

The foregoing discussion indicates the complexity of the relation existing between the composition of the spray solution, the temperature, and those factors primarily influencing evaporation. The latter factors have not been accurately measured and evaluated. The only way in which they have been given consideration here has been in delaying the application until after sundown. An accurate determination of their influence is needed if more consistent results are to be obtained. These determinations constitute a part of the work which is in progress.

Though the method which has been described for killing deep-rooted perennial weeds with an acid arsenical solution has been developed to such a degree that one is reasonably sure of satisfactory results provided all controlling factors are carefully considered, it should be emphasized that complete eradication is seldom, if ever, accomplished. Not all plants in a field can be in the proper condition at any one time. Those in the centers of heavily infested areas will have less water available than those bordering on uninfested regions. Often lateral roots extending into uninfested soil will be killed for some distance from the main root, but, becoming independent, will send up shoots and establish themselves as new plants. Morning-glory roots are often cut by gophers at various depths, from which they are able to send up new shoots and re-establish themselves. Insect injury often leads to a premature drying of foliar organs, and these plants are little affected by the spray solution.

One should further note that the problem of eradicating an old, established stand of almost any perennial weed involves more than the elimination of existing plants. Ridding the land of seeds remains as the most vital step if reinfestation is to be avoided. Experiments in progress indicate that a stand of morning-glory may, with proper handling, be sufficiently matured for effective treatment before viable seeds have been produced. Seeds which are green at the time of treatment are apparently killed by the arsenic compounds and fail to germinate.

Almost all stands of morning-glory, however, have a thorough infestation of the soil with seed from previous years, which must be germinated and killed before the danger is eliminated. Probably the best method for controlling the seedlings and the few old plants that come back after spraying is to cultivate thoroughly and weed-cut about twice after each irrigation, or each spring on unirrigated land. On the other hand, the plants may be allowed to mature and then sprayed; but this

method is more expensive and less certain than weed cutting. Further work must be done on this phase of the problem before definite recommendations can be made.

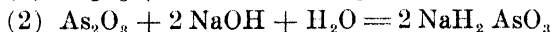
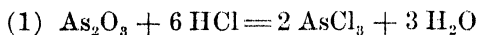
Because of the abundance of wild morning-glory (*Convolvulus arvensis*), the experiments described in this paper have been performed on that plant. A few tests on other weeds indicate that at least two more, alkali mallow (*Sida hederacea* Dougl.) and Russian knapweed (*Centaurea repens* L.), are equally susceptible to this type of treatment, if not more so. There is little hope of controlling the perennial grasses by this spray, for it is practically impossible to wet them with an aqueous solution and get the necessary intimate contact with the plant. Other flat-leaved plants that develop late enough in the season to deplete the soil moisture should lend themselves, however, to this treatment.

One need fear no permanent injury to the soil from the use of this type of spray. The small amount of arsenic trioxide used is insignificant in comparison with the mass of soil involved and seems to stimulate rather than inhibit the growth of subsequent crops. Experiments in soil sterilization indicate that applications of two hundred to three hundred pounds of sodium arsenite per acre have little effect upon subsequent crop growth. Evidently, then, many applications of the acid arsenical described above would be necessary to cause any permanent harm.

### PREPARATION OF THE SPRAY SOLUTION

For practical field use one must have a convenient method of preparing the spray solution. Few methods have been described <sup>(3, 5, 6)</sup> for the preparation of an acid arsenical solution for herbicidal purposes; and those few entail the use of arsenic pentoxide or arsenic acid, less toxic and more expensive forms than the common trioxide. During the experimental work just presented, a simple, practical method was developed which should prove useful for almost any scale of operation.

Arsenic trioxide is amphoteric and will react according to the following equations:



A solution may be made according to the first reaction by refluxing the arsenic trioxide in concentrated hydrochloric acid. The second reaction, being exothermic, goes very rapidly when only a small quantity of water is used.



As the spray solution should have an acid reaction, it would seem illogical to use alkali as an agent in dissolving the arsenic (equation 2). Probably for this reason, acid arsenicals previously made from arsenic trioxide have been produced by the costly process of refluxing with acid (equation 1). When one considers the problem quantitatively, however, and determines the cost of the chemicals used, it is obvious that the acidification of the alkaline solution, after it has been diluted to field strength, is by far the more practical method.

Because the alkali used in dissolving the arsenic must be neutralized in the final solution, it should be kept at a minimum in preparing the stock solution. A series of empirical tests with the commercial chemicals has shown that a solution containing 4 parts by weight of arsenic trioxide, 1 part by weight of sodium hydroxide, and 3 parts by weight of water is permanent and convenient to use as a stock solution. This forms a solution of sodium acid arsenite, in which the amount of sodium hydroxide is small; hence but little sulfuric acid is wasted in its neutralization. Five hundred gallons of spray solution containing 0.5 per cent arsenic trioxide would contain only 5 pounds of caustic soda, and about an equal quantity of sulfuric acid is required to neutralize it. The cost of these is negligible. Laboratory tests have shown that the small amount of sodium sulfate formed is entirely inert and in no way affects the action of the acid or arsenical. This quantity of sodium arsenite solution suffices for one acre of morning-glory, and previous data (table 2) indicate that if sulfuric acid to a concentration of 1.0 N (approximately 5 per cent by weight) is incorporated an effective herbicide will be produced.

Where only a few acres are to be sprayed, the necessary solution can be made up and handled with the equipment available on most farms. The stock solution of the arsenical does not affect iron containers; and though 1.0 N sulfuric acid is very corrosive to metal, when mixed with the necessary sodium arsenite it reacts only slowly with iron. Equipment designed to handle this solution in large quantities and over extended periods of time should be made of bronze or brass. Wooden tanks are not injured.

Nozzles throwing a flat, fan-shaped spray have given good results; the pressure should not exceed 100 pounds per square inch. An effort should be made to build up an excess of the solution on the foliage since the extent of distribution within the plant depends largely upon the volume of solution available.

As arsenic compounds are extremely poisonous, neither the dry arsenic trioxide dust nor the fumes from the stock solution or spray mist should be inhaled. The hands, the face, and, especially, the eyes should

be protected from both arsenicals and sulfuric acid; and livestock must be kept away from the chemicals, the solutions, and the sprayed vegetation. Sulfuric acid should be handled only by persons familiar with its properties. A bucket containing saturated bicarbonate of soda solution should be available at all times for washing the face or hands in case of an accident during the mixing or application of the spray solution.

## SUMMARY

A mechanism, dependent upon a water deficit in the plant, has been described to account for the movement of arsenicals from the sprayed foliage into the underground root system.

The most dilute arsenical solution giving effective control in field plots contained 0.5 per cent  $\text{As}_2\text{O}_3$  by weight. Higher concentrations up to 4 per cent seemed not significantly more effective.

An arsenical spray solution of acid reaction seems most effective in the field. The lowest effective concentration of the acid was apparently 1.0 N.

From late afternoon until midnight has been advocated as the best time to use this type of spray. The data given confirm this recommendation.

For best results the soil in which the plants are growing should not be excessively moist. There should be a full development of foliage.

Application of water to the dead foliage on the morning after spraying increased the percentage of plants killed.

The stock arsenical solution is prepared by mixing dry 4 parts by weight of  $\text{As}_2\text{O}_3$  and 1 part by weight of NaOH, adding 3 parts by weight of water and stirring until dissolved.

The spray solution is made by diluting 1 part by weight of the stock arsenical solution with 100 parts by weight of water, mixing thoroughly, and then adding, with constant stirring, 5 parts by weight of concentrated  $\text{H}_2\text{SO}_4$ .

About 500 gallons of this spray solution will be needed in treating an acre of morning-glory. Weeds having more abundant top growth require more solution.

A nozzle giving a fan-shaped spray, operating at 100 pounds pressure, has proved satisfactory.

The seedlings which come up after irrigation or during the spring after spraying on unirrigated land should be killed by cultivation or spraying.

No permanent injury to the soil results from this type of treatment.

The arsenious oxide and concentrated sulfuric acid used in the preparation of the spray solution should be handled with great caution.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

APRIL, 1933

No. 10

## REPRODUCTION WITHOUT MALES IN ASEPTIC ROOT CULTURES OF THE ROOT-KNOT NEMATODE<sup>1</sup>

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Parthenogenesis and syngonism<sup>3</sup> are recognized phenomena among the Nematoda, yet in the root-knot nematode, *Heterodera marioni* (Cornu),<sup>4</sup> where males do occur, their function in reproduction has been taken for granted. Byars (1914), however, did suggest the possibility of parthenogenesis in this nematode. Gabriel (1926) assumed its probability because he found several generations in one gall.

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<sup>3</sup> Maupas (1900), Cobb (1918), and others, have found that in certain free-living nematodes the gonad of the female produces spermatozoa, which are stored in a seminal receptacle and fertilize the eggs which are produced subsequently.

<sup>4</sup> This nematode has long been known as *Heterodera radiculicola*; but, as Goodey (1932) points out, the original description of *Anguillula radiculicola* by Greeff (1872) obviously does not refer to the root-knot nematode. Cornu (1879), then, was the first to publish a name for the latter, which he called *Anguillula marioni*. His descriptions and figures, though inaccurate and incomplete, do apply unmistakably to the root-knot nematode.

The genus (or subgenus) *Caconema*, proposed by Cobb (1924), is founded on three points: the type of parasitism, the structure of the amphids, and the number of testes. None of these is a distinguishing generic character in the classification of nematodes. Plant-parasitic and free-living species are commonly contained within the same genus (Imperial Bureau of Agricultural Parasitology, 1932), e.g., *Anguillulina* and *Pathoaphelenchus* [now *Aphelenchoides* (Steiner, 1932)]. The amphids vary in position, size, and structure from species to species (Steiner, 1923). The reproductive organs also are highly variable, as was observed by Bütschli in 1874. According to Baylis and Daubney (1926), each of the following genera contains species with two testes and species with only one: *Diplogaster*, *Tylencholaimus*, *Monhystera*, *Oncholaimus*, *Linhomoeus*, *Cyatholaimus*, *Chromadora*, and *Laxus*, a genus "of doubtful status," in which Cobb (1894; 1914) himself combined the two types of males. That Baylis and Daubney do not consider the number of testes a significant taxonomic character is indicated by the fact that they make no mention of the point in their synopsis of many of the genera of Ascaroidea, including *Spilophora*, which may be added to the list on the authority of Bütschli (1874). In the descriptions for the other four orders, testes are mentioned for only one genus.

Reproduction without males is obviously a great advantage to a parasite which must develop and lay eggs in one isolated location. There is, however, no true evidence for the phenomenon without actual pedigrees of isolated females, for which controlled laboratory cultivation is necessary.

Berliner and Busch (1914) made preliminary attempts to raise the sugar-beet nematode, *Heterodera schachtii*, in seedlings in agar. Byars (1914) raised the root-knot nematode aseptically through one generation in seedling cultures.

## METHOD

The method of cultivation described in the present paper permits the easy observation of individual nematodes in a fairly healthy environment. Complicating factors are eliminated by keeping the cultures bacteriologically sterile. Exact observations can thus be made on many phases of the life history.

Earliana tomato is used as the host plant. Seeds are disinfected with calcium hypochlorite (Wilson, 1915), and allowed to germinate in agar plates. The seedlings are transferred to fresh plates of a plant-nutrient medium.<sup>5</sup> One seedling is planted in each plate just before the agar sets, and for most of the cultures one nematode in the larval stage is placed beside the root tip. For the multiple infestations an egg mass was placed near the seedling, which was later found to harbor from 1 to 10 or more developing nematodes. Some larvae have difficulty in penetrating the root, but with good material galls appear in from one to five days, according to the temperature.

The plates can be kept for observation for a week or two, but better growth is obtained if the infested host plant, or part of its root, is transferred to a test tube containing Pfeffer's solution. A few grains of sand are added to each tube for a possible value in aeration.<sup>6</sup> The tubes can be kept sterile for weeks or months, and incubated as desired.

For obtaining uncontaminated larvae, the most practicable source of material is an infested potato. Here the egg masses are protected by a tough brownish case, which appears to be a shell of crushed plant tissue possibly impregnated with the oöthecal secretion discharged by the

<sup>5</sup> An agar medium is made with Pfeffer's solution (Robbins, 1922), modified by the substitution of ferric tartrate for ferric chloride (Hoagland, 1919) and by the addition of boron (Sommer and Lipman, 1926). The following formula was used:  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 4.0 grams;  $\text{KH}_2\text{PO}_4$ , 1.0 gram;  $\text{KNO}_3$ , 1.0 gram;  $\text{KCl}$ , 0.5 gram;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0 gram; ferric tartrate, trace; borax, 1:1,000 solution, 5 cc; glucose, 120 grams; agar agar, 60 grams; distilled water, 6,000 cc.

<sup>6</sup> Method used by G. Thorne.

nematode before egg-laying. The cases can be dissected out unbroken from the tuber. If they are originally free from contamination, a rinse of 3 to 5 minutes in hydrogen peroxide (c.p., full strength, i.e., 3 per cent) will clean them of any external organisms acquired during the manipulation, which need not be too carefully aseptic. From the peroxide, the egg masses are placed directly on agar plates, where any chance contamination can be discovered. Egg masses from tomato galls have also been used. They are considerably less convenient because the protecting cases are more fragile. It is also difficult to clean contaminating soil from the small galls.

The first larvae to emerge in the plates are those which have hatched some time earlier and lain packed, inactive, within the egg case. Later individuals are evidently newly hatched from the eggs. They wander on or through the soft agar and can be handled conveniently with a pointed splinter of bamboo, as used in Thorne's laboratory. A supply of splinters can be sterilized in test tubes in the hot-air oven.

### ABILITY OF LARVAE TO ENTER ROOTS

In view of the abundance of nematode galls in the field, it was most surprising to meet with considerable difficulty in obtaining galls in cultures, even on healthy growing seedlings. A great difference in the ability of nematodes to penetrate plant tissue was observed in different lots of larvae.

In an attempt to analyze this behavior, over 8,000 larvae have been tested. It is seen in table 1 that larvae raised in root cultures are able to enter fresh roots readily, while an initial infestation from field material was obtained less often. In the itemized daily records, infestation by larvae from cultures has been as high as 90 and 100 per cent of the individuals tested. When larvae hatched from a poor root were active enough to use for  $F_1$  cultures, they were as successful in entering the new host as were larvae from a better environment. By contrast, some lots of larvae from potato gave no galls, and only a few of the others gave 50 per cent infestation. Larvae from tomato gave 0 to 30 per cent.

There is no indication that external conditions affect the situation. It appears to be in the nature of some lots of larvae to enter the root tip readily and of other lots to find difficulty in penetration. Several possible explanations have been tested and discarded.

The first suggestion was that there might be a condition of dormancy of the nematodes in a dormant potato, or else that the larvae there were somewhat older and weaker than the normal. Egg masses were then taken

fresh from tomato roots growing in pots. There is no reason to assume that dormancy occurs under greenhouse conditions; yet the proportion of galls formed by these larvae was even lower than by those from potato.

This comparison also answers Steiner's (1925) suggestion that a race of nematodes accustomed to potato might resist a change of host. On the contrary, larvae from potato make more galls in tomato seedlings than do those from greenhouse tomato roots (table 1). The larvae from one lot of potatoes<sup>7</sup> in particular showed a high power of infestation. These nematodes had been in potato only two or three generations, however. Their previous host was tomato.

TABLE 1  
ROOT PENETRATION BY LARVAE FROM DIFFERENT SOURCES

Material	Larvae tested	Per cent infestation
Potato (3 lots various ages)	1 810	6 0
Tomato (fresh from greenhouse)	1,599	4 0
Potato No. n464	91	27 5
Tube cultures (mostly from isolated females)	4,933	54 2

There is no reason to suspect the peroxide treatment of weakening the eggs or larvae. All the egg masses from No. n464 received this treatment, as did also a few of the galls from cultures. Material so treated gave infestations up to 90 per cent.

Cultured larvae appear exceptionally large and vigorous. The absence of other organisms cannot account for this because the egg masses within an otherwise healthy potato are equally aseptic.

Only two observations seem to have significance: (1) There is an apparent stimulation of larvae which have hatched in a liquid. This has been observed in sterilized Pfeffer's solution, and in tap and distilled water. (2) Larvae hatched and "dormant" within the egg mass in potato, and larvae kept in tubes of any liquid longer than a few weeks, seem to

<sup>7</sup> In December, 1928, potatoes were received from Goodyear's Bar, in the Sierra Nevada foothills. Though stunted by nematode attack, the tubers seemed to have resisted the invaders by walling off many of them with heavy brown tissue. Potatoes grown from this strain failed to carry on the resistance. Small tubers of the third generation were harvested from one can (No. n464) in November, 1929, after four months' growth outdoors in Berkeley. The potatoes were kept in the laboratory all winter, and dissected April 17, 1930. Nematodes were found in only 5 of the 28 tubers, but one of these was packed with nematodes in all stages of development. It was a potato only  $\frac{3}{4}$  inch in diameter. Over a thousand females, large and small, were counted with the naked eye. Three fourths of the tissue of the tuber showed a watery degeneration from the heavy infestation. The other four tubers had small watery spots packed with nematodes.

be less active in infesting a root. Even cultured larvae become weakened if kept too long in their sterile tubes. Their reserve energy<sup>8</sup> is used up in fruitless activity. In the field, larvae are believed to survive 15 months' starvation (Bessey, 1911). Dryness, temperature, and aeration may have much to do with their survival, and there may in reality be only a small proportion of a population which is able to start the new infestation.

## NUTRITION AND DEVELOPMENT OF NEMATODES IN ROOT CULTURES

Among the seedlings used as host plants, there occurred wide differences in the vigor of root growth. In general, the development of the nematode corresponded to the condition of the host. This statement may seem obvious, yet it becomes significant as the progressive effects of malnutrition are seen, culminating apparently in an alteration of the sex ratio.

In primary cultures, when only one nematode was used for each plant, 50 per cent of the population completed the life cycle from larva to larva ("per cent hatch," table 2), but when one seedling had to support several parasites, only 10 per cent completed the cycle. Again, in single-nematode cultures, only 12 per cent failed to develop as far as sexual differentiation, while in multiple infestations, 61 per cent remained undifferentiated.

A more extreme case of undernourishment occurred in long-unopened cultures ("secondary galls," table 2). Some of the F<sub>1</sub> larvae in tubes attacked the root of the host plant from which they had hatched. The plants had been weakened by age and artificial conditions to the point where they no longer provided healthy growing tissue for their parasites. These secondary galls turned brown, and the effect of starvation on the nematodes was conspicuous: 79 per cent remained undifferentiated, and only 2 of the 20 females contained ova, which failed to develop.

Cultural conditions were not always unfavorable for the development of nematodes. In culture No. 2391, one female laid 1,998 eggs, counting 1,406 larvae and 592 unhatched eggs, the highest number of eggs reported for a single female.

Development from larva to larva is possible even in some very unfavorable roots, but the number of eggs laid, and the number, size, and

<sup>8</sup> Godfrey, Oliveira, and Gittel (1933) have demonstrated the food material stored by *Heterodera marioni* as layers of fat in the cells of the intestine.



vigor of larvae are subject to nutritional conditions. Galls can be formed in bits of root tips as short as 5 mm. These conditions are far from normal, but the life cycle has been completed in several such cultures.<sup>9</sup>

From time to time a gall has been found in which the nematode was apparently too weak to lay her eggs, or where external pressure left no room for an egg mass. Larvae inside the body of the female appeared healthy. A similar situation occurs in potatoes, where sometimes the last eggs are not laid by an exhausted female. Nagakura (1930) describes such a female as a "*Brutkapsel*"; it may be also the "cyst form" of Jones (1932).

TABLE 2  
SEX AND AMOUNT OF DEVELOPMENT OF NEMATODES IN CULTURES

Type of gall	Infestation	Root growth	Sex undifferentiated*	Males	Females			Total number of nematodes	Per cent undifferentiated	Per cent males†	Per cent hatch‡	Per cent infestation by F <sub>1</sub> larvae§
					No eggs or no hatch		Eggs hatched					
					Insufficient time	Sufficient time						
Primary	Single	Poor	63	6	49	94	95	307	20.5	2.5	37.7	58.5
		Fair	61	2	130	115	158	466	13.1	0.5	47.3	54.4
		Good	39	1	117	158	285	600	6.5	0.2	59.1	53.7
		All roots	163	9	296	367	538	1,373	11.9	0.7	50.4	54.2
	Multiple	Poor	41	4	.....	18	7	70	58.6	13.8	10.6	.....
		Fair	52	6	.....	14	5	77	57.5	24.0	7.0	.....
		Good	3	0	.....	4	3	10	30.0	0.0	30.0	.....
		All roots	96	10	.....	36	15	157	61.1	16.4	10.2	.....
Secondary	Multiple	Old	174	26	.....	20	0	220	79.1	56.5	0.0	.....

\* These nematodes were allowed sufficient time to complete the life cycle.

† Per cent males is the ratio of males to the total adult population.

‡ Per cent hatch is the percentage of all the nematodes in any series (excluding males, and the females which had insufficient time for development) which completed the life cycle.

§ Per cent infestation is based on the number of larvae tested. Only a few subcultures were made with F<sub>1</sub> larvae from multiple infestations.

## PEDIGREES

The conditions of the method outlined above are such that, if each plate is planted with only one larva, the fact of reproduction without males can be clearly established. In most cases the unmated females in cultures laid viable eggs. When these eggs hatched, subcultures were started using the aseptic F<sub>1</sub> larvae, one to a plate.

<sup>9</sup> Five out of 12 cultures gave the complete cycle. Of the other 7 individuals, 1 remained immature, 2 were males, and 4 were females.

TABLE 3

REPRODUCTION BY ISOLATED FEMALES IN ONE FAMILY OF THE ROOT-KNOT NEMATODE

First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth
		1522	2064		3703	4784		6002			
		1529			3705	4788		6003			
		1600			3707	4792	5474	6009	6832, δ		7786
		1601	2215	48, 29*	3720	4795		6117	90**		7798
		1603	3455		3727	4799		6119			7799
		1604	3458		3800	4856	6699	6121			8174
		1606	3465			4866		6124			
		1617	3466	2272		4867	6350	6125			
		1618		2273		4873		6131	6536	7266	
		1620	3391	3439		4874		6132	6580	7197	7939, δ
		1623	3392	3445		4888		6135	7374	7945	7946
		1624		3451		4891		6137		7951	7952
		1626	16*	3451		4894		6138		7953	7954
		1627		3567		4905	6333	6139		7955	7956
		1628	1654	3572		4906		6140		7957	7958
		1629	1657	3575		4907		6141		7959	7960
		1630	1658	3579	26*	4908		6142		7961	7962
		1631	1661	3581		4909		6143		7963	7964
		1632	1664	3582		4910		6144		7965	7966
		1633	1665	3583		4911		6145		7967	7968
		1634	1666	3584		4912		6146		7969	7970
		1635	1667	3585		4913		6147		7971	7972
		1636	1668	3586		4914		6148		7973	7974
		1637	1669	3587		4915		6149		7975	7976
		1638	1670	3588		4916		6150		7977	7978
		1639	1671	3589		4917		6151		7979	7980
		1640	1672	3590		4918		6152		7981	7982
		1641	1673	3591		4919		6153		7983	7984
		1642	1674	3592		4920		6154		7985	7986
		1643	1675	3593		4921		6155		7987	7988
		1644	1676	3594		4922		6156		7989	7990
		1645	1677	3595		4923		6157		7991	7992
		1646	1678	3596		4924		6158		7993	7994
		1647	1679	3597		4925		6159		7995	7996
		1648	1680	3598		4926		6160		7997	7998
		1649	1681	3599		4927		6161		7999	8000
		1650	1682	3600		4928		6162		8001	8002
		1651	1683	3601		4929		6163		8003	8004
		1652	1684	3602		4930		6164		8005	8006
		1653	1685	3603		4931		6165		8007	8008
		1654	1686	3604		4932		6166		8009	8010
		1655	1687	3605		4933		6167		8011	8012
		1656	1688	3606		4934		6168		8013	8014
		1657	1689	3607		4935		6169		8015	8016
		1658	1690	3608		4936		6170		8017	8018
		1659	1691	3609		4937		6171		8019	8020
		1660	1692	3610		4938		6172		8021	8022
		1661	1693	3611		4939		6173		8023	8024
		1662	1694	3612		4940		6174		8025	8026
		1663	1695	3613		4941		6175		8027	8028
		1664	1696	3614		4942		6176		8029	8030
		1665	1697	3615		4943		6177		8031	8032
		1666	1698	3616		4944		6178		8033	8034
		1667	1699	3617		4945		6179		8035	8036
		1668	1700	3618		4946		6180		8037	8038
		1669	1701	3619		4947		6181		8039	8040
		1670	1702	3620		4948		6182		8041	8042
		1671	1703	3621		4949		6183		8043	8044
		1672	1704	3622		4950		6184		8045	8046
		1673	1705	3623		4951		6185		8047	8048
		1674	1706	3624		4952		6186		8049	8050
		1675	1707	3625		4953		6187		8051	8052
		1676	1708	3626		4954		6188		8053	8054
		1677	1709	3627		4955		6189		8055	8056
		1678	1710	3628		4956		6190		8057	8058
		1679	1711	3629		4957		6191		8059	8060
		1680	1712	3630		4958		6192		8061	8062
		1681	1713	3631		4959		6193		8063	8064
		1682	1714	3632		4960		6194		8065	8066
		1683	1715	3633		4961		6195		8067	8068
		1684	1716	3634		4962		6196		8069	8070
		1685	1717	3635		4963		6197		8071	8072
		1686	1718	3636		4964		6198		8073	8074
		1687	1719	3637		4965		6199		8075	8076
		1688	1720	3638		4966		6200		8077	8078
		1689	1721	3639		4967		6201		8079	8080
		1690	1722	3640		4968		6202		8081	8082
		1691	1723	3641		4969		6203		8083	8084
		1692	1724	3642		4970		6204		8085	8086
		1693	1725	3643		4971		6205		8087	8088
		1694	1726	3644		4972		6206		8089	8090
		1695	1727	3645		4973		6207		8091	8092
		1696	1728	3646		4974		6208		8093	8094
		1697	1729	3647		4975		6209		8095	8096
		1698	1730	3648		4976		6210		8097	8098
		1699	1731	3649		4977		6211		8099	8100
		1700	1732	3650		4978		6212		8101	8102
		1701	1733	3651		4979		6213		8103	8104
		1702	1734	3652		4980		6214		8105	8106
		1703	1735	3653		4981		6215		8107	8108
		1704	1736	3654		4982		6216		8109	8110
		1705	1737	3655		4983		6217		8111	8112
		1706	1738	3656		4984		6218		8113	8114
		1707	1739	3657		4985		6219		8115	8116
		1708	1740	3658		4986		6220		8117	8118
		1709	1741	3659		4987		6221		8119	8120
		1710	1742	3660		4988		6222		8121	8122
		1711	1743	3661		4989		6223		8123	8124
		1712	1744	3662		4990		6224		8125	8126
		1713	1745	3663		4991		6225		8127	8128
		1714	1746	3664		4992		6226		8129	8130
		1715	1747	3665		4993		6227		8131	8132
		1716	1748	3666		4994		6228		8133	8134
		1717	1749	3667		4995		6229		8135	8136
		1718	1750	3668		4996		6230		8137	8138
		1719	1751	3669		4997		6231		8139	8140
		1720	1752	3670		4998		6232		8141	8142
		1721	1753	3671		4999		6233		8143	8144
		1722	1754	3672		5000		6234		8145	8146
		1723	1755	3673		5001		6235		8147	8148
		1724	1756	3674		5002		6236		8149	8150
		1725	1757	3675		5003		6237		8151	8152
		1726	1758	3676		5004		6238		8153	8154
		1727	1759	3677		5005		6239		8155	8156
		1728	1760	3678		5006		6240		8157	8158
		1729	1761	3679		5007		6241		8159	8160
		1730	1762	3680		5008		6242		8161	8162
		1731	1763	3681		5009		6243		8163	8164
		1732	1764	3682		5010		6244		8165	8166
		1733	1765	3683		5011		6245		8167	8168
		1734	1766	3684		5012		6246		8169	8170
		1735	1767	3685		5013		6247		8171	8172
		1736	1768	3686		5014		6248		8173	8174
		1737	1769	3687		5015		6249		8175	8176
		1738	1770	3688		5016		6250		8177	8178
		1739	1771	3689		5017		6251		8179	8180
		1740	1772	3690		5018		6252		8181	8182
		1741	1773	3691		5019		6253		8183	8184
		1742	1774	3692		5020		6254		8185	8186
		1743	1775	3693		5021		6255		8187	8188
		1744	1776	3694		5022		6256		8189	8190
		1745	1777	3695		5023		6257		8191	8192
		1746	1778	3696		5024		6258		8193	8194
		1747	1779	3697		5025		6259		8195	8196
		1748	1780	3698		5026		6260		8197	8198
		1749	1781	3699		5027		6261		8199	8200
		1750	1782	3700</							

TABLE 4  
REPRODUCTION BY ISOLATED FEMALES IN  
FAMILIES FROM VARIOUS SOURCES

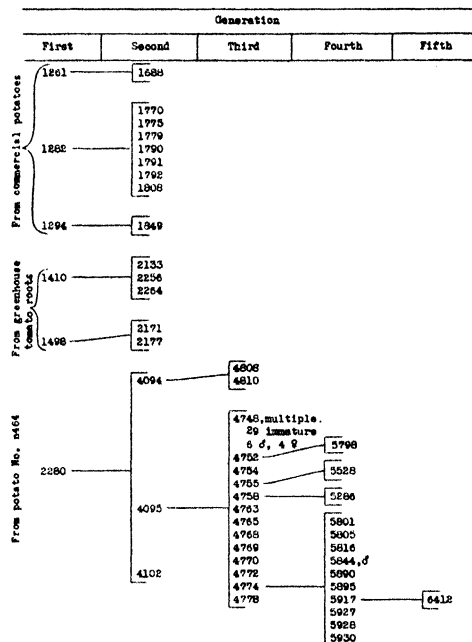


TABLE 5  
REPRODUCTION AND INCIDENCE OF MALES IN FIRST-GENERATION CULTURES OF  
NEMATODES FROM VARIOUS SOIL POPULATIONS

Source of larvae		Infestation	Males	Females: sufficient time		Per cent males
				No eggs or no hatch	Eggs hatched	
Commercial potatoes	California, 1929	Single	3	9	17	10.3
		Multiple	2	6	2	20.0
	Nevada, 1929	Single	0	4	9	0.0
		Multiple	0	11	4	0.0
	California, 1930	Single	0	0	5	0.0
Potato No. n464.....		Single	0	1	11	0.0
Greenhouse tomato roots.....		Single	0	3	25	0.0
		Multiple	1	13	3	5.9

One family of the root-knot nematode has been carried through 12 generations by repeated isolations. The pedigree is given in table 3, in which individual nematodes in primary galls are represented by culture numbers. The original parent of the family, No. 408, was a nematode from a commercially grown potato ("California, 1929," in table 5). Each culture number, including No. 408 but excepting those designated as males and one culture designated as multiple, stands for an isolated female with life history complete from larva to larva. Females with incomplete life histories have been omitted from the pedigree, except that in cases where males would otherwise appear to predominate, the number of females with incomplete life histories in primary or in secondary galls is also given, marked with a double or a single asterisk. The occurrence of males in secondary galls is indicated by the number of males, marked with an asterisk.

Additional pedigrees are shown in table 4, one family with 5 complete generations and five with 2 complete generations of isolated females. The 6 nematodes which form the first generation of these 6 families respectively were taken in the egg stage from commercial potatoes ("California, 1929," in table 5), from the home-grown potato, No. n464, and from tomato galls grown in the greenhouse.

Table 5 shows the first generation of nematodes in test-tube cultures started from field or greenhouse populations. It includes the 7 females of the first generation which appear also in tables 3 and 4, making a total of 67 unmated females whose eggs hatched normally, sufficient to show that reproduction without males occurred readily in the first generation of the 5 populations tested. There was a high percentage of males from the first potato population, but because the 5 males were all in poor roots, their occurrence is not inconsistent with the behavior of other material.

### OCCURRENCE OF MALES IN CULTURES

In single-nematode cultures (table 2), there were only 9 males to 1,201 females. There were 10 males to 51 females in the multiple primary infestations, and a still higher ratio of males in the secondary galls, which contained 26 males to only 20 females. Some of the males were less than half the normal length.

The difficulty of development in the more heavily infested roots can be seen by comparing the three types of infestation in table 2. Conditions of nutrition were most favorable in single primary infestations, less so in multiple primary infestations, and conspicuously unfavorable

in the secondary infestations, which were also multiple. Accordingly, the percentage of larvae which were unable to reach the stage of sexual differentiation increased through the series, while the percentage of females whose eggs were able to hatch decreased from single to multiple primary infestations, and in the secondary infestations there were no eggs laid. The increase in percentage of males developed in the cultures is correlated with the increasingly adverse conditions of nutrition.

There is no indication that this is merely a female-producing strain of the root-knot nematode. The occurrence of males appears to follow the same pattern in the different populations used. Table 2 shows 19 males in primary and 26 in secondary infestations. This is the total number of males developed in all cultures. Tables 3, 4, and 5 give their positions in the families, where no genetic relation can be found. The 6 males in original infestations from potato or tomato material (table 5) occurred in poor roots, and unfavorable cultural conditions also prevailed in the secondary galls, which provided by far the highest percentage of males.

The cultures of the sugar-beet nematode made by Berliner and Busch (1914) also produced males in host roots which apparently made little growth. A male developed in a decaying bit of root, in which a second larva failed to grow at all. The male was perfectly formed but abnormally short, because of undernourishment. Another male is described, but only one female, "almost mature."

Although it was not the purpose of this investigation to deal with genetic problems, it does not seem unreasonable to suggest that conditions of nutrition may be important enough in the early stages of development to change the physiological balance of certain individuals. This point of view has been recognized by Babcock and Clausen (1927), who conclude their chapter on sex determination as follows:

The thesis that sex under normal conditions is determined at the time of fertilization is supported by abundant cytological and genetic investigations. This theory is not inconsistent with the view that the differentiation of sex during development depends upon a complex series of interactions between factors located in the sex chromosomes and autosomes; nor with the observation that internal secretions of the gonads and other glands may play an important part in the process. Nor is it inconsistent with the view that the sexes may be characterized by differences in metabolic rate, nor that changes in the metabolic rate or alterations in the type of internal secretions circulating in the blood during development may go so far as to reverse completely the sex determined by the original zygotic constitution.

It may be interesting to note that Allen (1932) compares hermaphroditism in animals with the situation in monoecious plants, where "the potentialities for the production of the characters of both sexes must

reside in each individual," and the expression of either set of characters may be favored or inhibited by additional factors, either genetic or environmental.

There is a temptation to ask whether fertilization may not be necessary or helpful at the very time the males appear, i.e., when a population has been weakened by malnutrition; but that would be to plead the direct influence of nutrition on sex. Furthermore, it is not known whether these or any of the males are functional, nor whether fertilization may be haphazard. Maupas (1900) considers that the few males which occur in parthenogenetic species are only atavistic, and lack the mating instinct.

Any conclusions must depend, of course, on a study of the germ cells. In *Ascaris* (Edwards, 1910) and in some other nematodes (Gulick, 1911) sex determination is of the XY type. There is no information on the chromosome behavior of *Heterodera*, nor is there cytological evidence on the question of parthenogenesis vs. syngonism.<sup>10</sup>

#### OCCURRENCE OF MALES IN ROOTS GROWN IN SOIL

Occasional lots of field material have been found with a preponderance of males. This condition was conspicuous in strawberry roots from San Luis Obispo County. Nearly every gall contained 1 or 2 males, and some showed 5 and 6 males to 1 female. These males seemed unusually short and broad. Strawberry roots obtained from the same field a month later yielded fewer males.

The value of a census taken by dissection of galls and egg masses is limited because of the free traveling of the males. However, when the question was raised, counts were made by dissection of various lots of material. They are given in table 6 as a matter of general interest. There is no correlation between the different percentages, and more cases would be needed before conclusions could be drawn.

The lack of males was not observed in seedlings grown in jars of soil for temperature experiments in 1925 and 1927. The roots were dissected before the males had time to wander, and data are thus more complete than for random field collections. But because the small seedlings were heavily attacked from the beginning, neither the plants nor the

<sup>10</sup> Atkinson (1889) found spermatozoa in the oviducts of the root-knot nematode. Nagakura (1930) describes and figures the seminal receptacles, in which he saw many spermatozoa. He states that dead males were frequently found among the eggs in the oötheca. These reports raise more questions for the cytologist to answer. Von Sengbusch (1927) observed copulation twice in experiments with the sugar-beet nematode, and found spermatozoa in most of the females examined. Considering the abundance of males in the sugar-beet nematode (Molz, 1920), it is very possible that normal mating may occur in that species.

nematodes had a fair chance to develop normally. For completeness of count, the tube cultures are of course the most accurate of all, and it is there that the fewest males are found.

In the 1931 temperature experiments, in soil between 12° and 26° C, only 8 males were found, mostly in decaying galls, while there were 992 females, in roots of every degree of vigor. However, in 3 cages at 28° C, there were 74 males to 354 females. The air bath used in this experiment did not favor a healthy growth of the host plants. The soil dried rapidly.

TABLE 6  
MALES FOUND IN ROOTS GROWN IN SOIL

Host plant	Source	Males	Females	Per cent males
Strawberry	San Luis Obispo County .....	48	29	62.3
	San Luis Obispo County, second shipment .....	27	72	27.3
	Carlsbad, California .....	1	36	2.7
	Old roots in formalin .....	1	25	3.8
Potato	From Nevada, 1929 .....	0	41	0.0
	From California, 1930 .....	0	24	0.0
Tomato	Fresh from greenhouse .....	19*	178	9.6*
	Later generations of strawberry strain from Carlsbad .....	6	23	20.7
	Later generations of strawberry strain, second shipment from San Luis Obispo County .....	22	329	6.3
	Temperature experiments { 1925 .....	15	89	14.4
	(not at extreme temperatures) { 1927 .....	70	1,014	6.5
	{ 1931 { 28° C .....	74	354	17.3
	{ Other cages .....	8	992	0.8
Totals	.....	291	3,206	8.3

\* Of the 19 males, adult or developing, noted above from tomato, 10 were found in one large gall. The percentage of males is raised by this one gall.

The roots were too heavily infested with nematodes: 46 of the 74 males were developed in crowded galls. Temperature does not directly influence the sex ratio, for in experiments with 835 single-nematode cultures at all temperatures, including the biological extremes, there were only 5 males—all in poor roots. There were no males at all in the culture-solution experiments between 25° and 35°.

On two other occasions a "nest" of males has been found in one gall, an obvious case of overcrowding. One was a tomato root fresh from the greenhouse. Ten males were found in one large gall. In the 1925 temperature experiments, one gall contained 6 males and 2 young females.

There was crowding in tube cultures also. The 14 secondary galls in culture No. 2785 gave a count of 12 males, 3 immature females, and 9 individuals in earlier stages of development. In culture No. 4748 the

primary infestation was too heavy for good growth, and dissection of 6 galls showed 29 individuals in early stages, 6 males, and 4 females without eggs. These two cultures occurred in different families, as can be seen in tables 3 and 4.

Molz (1920) found that in the sugar-beet nematode the development of females was favored by a heavy nitrogen fertilization and by other conditions which promoted a vigorous growth of the host plants, and that a larger proportion of males appeared when the host plants were weakened for any reason. According to his figures, males of that species are usually more numerous than females.

Hornburg (1929) was able to raise the ratio of males of the sugar-beet nematode from 90 to 500 per 100 females, or to reduce it from 300 to 90 per 100 females. His treatment, watering the host plant with infusions of other plants or of seeds, did not affect the amount of infestation.

### SUMMARY

A method of obtaining uncontaminated larvae and of raising them in sterile seedlings is described. This method of cultivation lends itself to the detailed study of a variety of problems.

Larvae raised in root cultures were much more active than larvae from the field in entering growing seedlings in vitro. There was also a great variation in this respect among different lots of nematodes grown in soil, related in part but not wholly to the freshness of the larvae.

A healthy condition of the host plant is important for the development of its parasites.

Reproduction without males appears to be regular and normal for the root-knot nematode. One family has been carried through 12 complete generations by repeated isolations, and the same type of reproduction has been demonstrated in other families from various sources.

The occurrence of males is rare: only 0.7 per cent have been found in single-nematode cultures. Males appeared more frequently in old, unhealthy, or heavily parasitized roots. There were 16.4 per cent in multiple primary infestations, while in secondary infestations, also multiple, 56.5 per cent of the nematodes which were able to develop were males, a ratio of 130 males to 100 females. Observations are presented which suggest that in the field also males occur under adverse conditions.

### ACKNOWLEDGMENT

The writer appreciates the assistance of Professor W. B. Herms, who has been most helpful in the preparation of the manuscript and in discussion of the fundamental problems involved.



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DEVELOPMENT OF THE ROOT-KNOT NEMATODE  
AS AFFECTED BY TEMPERATURE

JOCELYN TYLER



# DEVELOPMENT OF THE ROOT-KNOT NEMATODE AS AFFECTED BY TEMPERATURE<sup>1</sup>

JOCELYN TYLER<sup>2</sup>

## INTRODUCTION

It is well known that invertebrate animals are dependent on the temperature of the environment for their vital activities. In the case of the root-knot nematode, *Heterodera marioni* (Cornu), this is a factor which must be considered in every phase of biological study. Since it has a direct influence on the rate of metabolism, it is obvious that it must also have an important bearing on problems to which it has not heretofore been applied, such as rate of travel through soil, rate of killing by chemicals, and rate of starvation in fallow fields, as well as on the amount of infestation and the damage done to crops, which have been investigated by Godfrey (1926) and by L. H. Jones (1932).

Temperature is not, however, the only factor influencing the rate of development of this nematode. Godfrey and Oliveira (1932) grew cowpea and pineapple plants side by side in the greenhouse. Yet under identical conditions, development to egg-laying took 35 days in pineapple and only 19 days in cowpea.

Baumacke (1922) has analyzed the effect of temperature on the sugar-beet nematode. His thesis is that the larva, which may normally survive in the soil for months without feeding, depends on a food reserve which it has stored up from the egg. When the soil is cool the larvae are fairly inactive, and the reserve will be used slowly. With higher temperatures, motion and also sense perception are accelerated, so that the larvae should be able to find host plants before the food reserves become exhausted. For the sugar-beet nematode he gives the optimum temperature as 25° C, with a maximum activity at 28°. This increased activity is an escape reaction, which should in nature assist the migration of the larva to a host root or to a cooler environment. If prolonged, it causes death by exhaustion as an indirect result of the high temperature.

In the present paper, it is proposed to analyze the reactions of the root-knot nematode to temperature, as nearly as possible apart from

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other factors. One of the principal objects has been to determine the shortest time for development at different temperatures. The conclusions may not hold good for all hosts, as Godfrey and Oliveira (1932) have demonstrated, but the tomato plant is a very favorable host and the minimum records obtained for it are probably not far from the minimums for most other plants.

## METHODS OF EXPERIMENTATION

Earliana tomato was used exclusively as the host plant. Infested roots were grown at controlled temperatures in three series of experiments, one in Pfeffer's solution and two in soil. Results were consistent throughout the three series.

The aseptic seedling cultures described in the foregoing paper (Tyler, 1933) made suitable material for temperature experiments. In order to study the behavior of individual nematodes, only one worm was used in a culture, and the time was recorded for each culture separately, starting at the first sign of gall formation, when the seedling was transplanted to a test tube containing Pfeffer's solution and placed in an incubator chamber.

In 1927, experiments were made with tomato plants growing in jars of soil, in a water bath patterned after the tanks installed at Johns Hopkins University (Livingston and Fawcett, 1920). The water was kept in continuous circulation around the jars by bubbling compressed air up from the bottom of the tank through a series of holes in a tube the length of each compartment. Battery jars were filled with infested soil from the greenhouse, subirrigated by the method of Jones and Tisdale (1921). When this soil had been brought to the desired temperature, nematode-free seedlings were transplanted into it and the records of the experiment were started. The soil temperature was guarded by an insulation of mineral wool (Jones and Tisdale, 1921). The tops of the plants were in the air of the room.

In 1931 plants were grown in the type of root cages described by Dean (1929), some in cold storage and others in an air bath heated by thermostatically controlled light bulbs, with air circulation supplied by a small electric fan. Records were started when a water suspension of active larvae was poured directly over the previously uninfested roots, where they grew along the glass at the side of the cage.

Standardized thermometers were kept in positions where they would show the actual temperatures of the roots. For soil experiments they were set into the soil at the depth of the galls. For culture-solution experiments they were placed in tubes containing tap water, two for

each basket of culture tubes. Charts of the root temperatures were made from thermograph records checked against the thermometer readings. A fluctuation of  $\pm 1^{\circ}$  C was unavoidable in some of the experiments. Any wider fluctuations were only temporary. Four or five accidents occurred in the different experiments, giving a deviation of  $3^{\circ}$  to  $12^{\circ}$ , but not reaching extreme temperatures. In most cases, the fluctuations were only a fraction of a degree beyond the limits established. The total time for both types of variation was never more than 20 per cent of the time of any of the experiments reported, and generally much less.

Moisture control was not considered necessary in the soil experiments, because this nematode thrives, especially in the gall, throughout the moisture range of good plant growth (Godfrey, 1926).

Artificial light was supplied for plants growing in soil. Some of the roots in culture solution grew as well with stem and leaves removed as did others with top parts intact.

#### METHODS OF MEASURING DEVELOPMENT

The stages of development from stage 3, the free-living larva, to stage 12, the full-grown female, are numbered in accordance with plate 1 of Bessey's paper (1911), which is reproduced in part in figure 1. After the nematode has reached stage 12, the gelatinous oöthecal secretion is extruded from the vulva, and somewhat later small ova can be found in the oviducts. The final stage for these experiments is the extrusion of the first eggs. The metamorphosis of the male is shown in stages 13, 14, and 15.

Each record shows the stage of development reached by one nematode in a certain number of days at a certain temperature. Since the galls were dissected for examination, only one observation could be made on each nematode, and the slower cases are open to several possible interpretations. There are individual differences in rate of development, as will be shown in connection with the rate of egg-laying. In some cases, however, development is undoubtedly arrested by unfavorable conditions, and this may have occurred some time before the examination, so that there is no way of determining when the recorded stage was reached except by comparison with other individuals at the same temperature. Allowance should also be made in soil experiments for delayed infestation. The minimum-time records are more significant because they give definite evidence of what really happened during the time of the experiment.

The time and temperature relations may be expressed in heat units. Each Centigrade degree above  $10^{\circ}$ , acting for one hour, is counted as



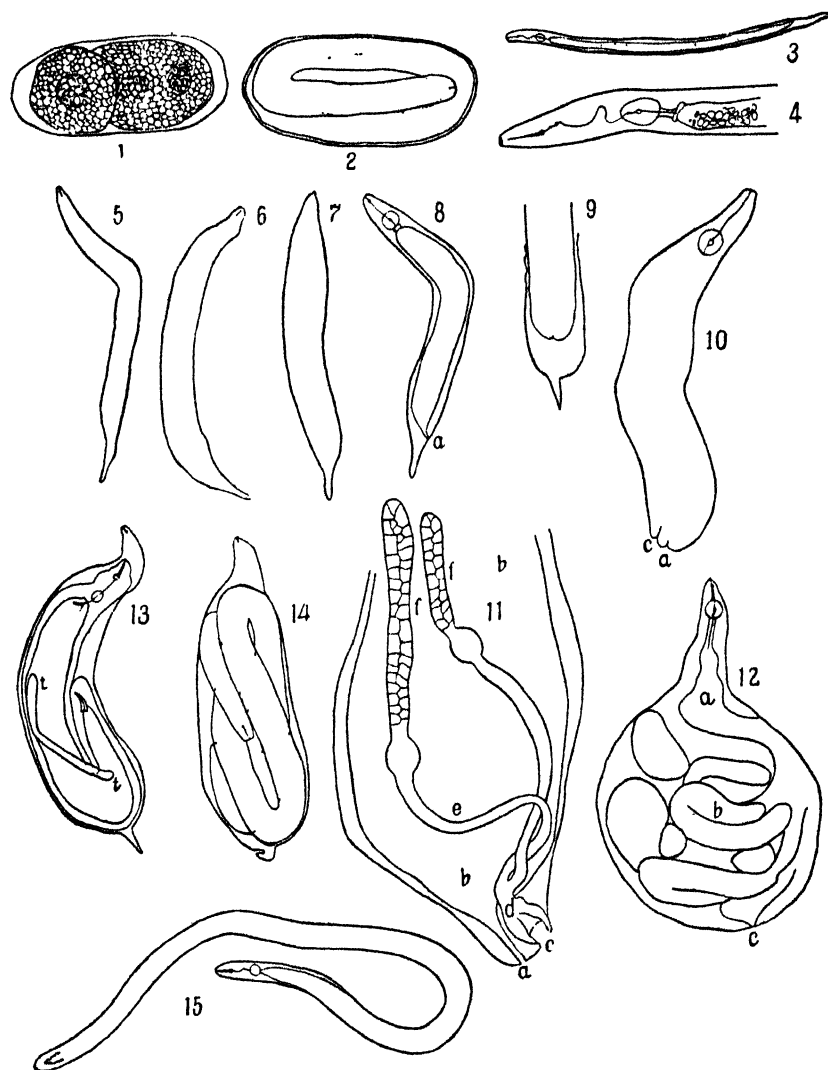


Fig. 1. Stages in the development of the root-knot nematode. [After Bessey (1911), by permission from the United States Department of Agriculture.] 1 and 2, Developing eggs. 3 and 4, Free living larva and its anterior portion. 5 to 8, Stages in growth of larvae. 9, Posterior portion of immature female just before molting, recognized by the old larval tail which has not been stretched along with the rest of the cuticle. 10, Female after molt: *a*, anus; *c*, vulva. 11, Posterior portion of young female. 12, Full grown female: *a*, alimentary canal; *b*, loop of oviduct; *c*, vulva. 13 and 14, Metamorphosis of male. 15, Free adult male.

one effective unit. A temperature of  $16^{\circ}$  for 24 hours is thus counted as  $24 \times 6 = 144$  units. Summation of these units gives a measure of the heat requirements, which are approximately constant for a given stage of the life history at any medial temperature, and which increase with succeeding stages. For example, the normal range was as follows, both in soil and in culture-solution experiments: 500 heat units for gall formation by larvae; 2,100–4,000 for development of a nematode from the appearance of a gall to stage 9; 3,700–6,000 to stage 10; 4,800–7,000 to stage 12; 6,300–7,500 to the formation of ova; 6,500–8,000 to the beginning of egg-laying; and roughly 5,000 more for the development and hatching of eggs. Shelford (1927) has pointed out several objections to the summation method. It has nevertheless a practical usefulness, and its inaccuracies are no greater than the variations shown by individual nematodes. It also emphasizes certain differences between mathematical expectations and experimental results, such as the amount of development at very low temperatures, and the different threshold relations of young and mature stages.

For any stage of development, the time and temperature records of individual nematodes at medial temperatures fall roughly into a parabolic curve (fig. 2), practically paralleling the curve of hour-Centigrade-degree units. A heat-unit curve can thus be used as a working basis for predicting the time required for development at any given temperature, within the medial range, and is useful in comparing records obtained at different temperatures.

Heat units were computed empirically from  $10^{\circ}$  C because  $11.5^{\circ}$ , the "a point" of the rate curve (fig. 3), was too high for many of the low-temperature experiments, while  $9^{\circ}$ , which is close to the true threshold temperature for early development at least, is too far from this curve. The threshold of development is below the parabola and the rate curve in any case (Krogh, 1914), and parabolas calculated from  $10^{\circ}$  are harmonious with the majority of the data from experimentation.

In cultures held at  $10^{\circ}$  or  $12^{\circ}$  C for a long period, considerable development may be shown where only a few hundred heat units are recorded. In such cases the threshold temperature is obviously below  $10^{\circ}$ . At these low temperatures the sum of heat units above  $9^{\circ}$  is often two or three times the number computed from  $10^{\circ}$ , although in brief exposures at higher temperatures the difference is negligible. In such special cases, the usual computation gives a false picture of the situation, and the rate of development can be better interpreted by calculating heat units from the threshold,  $9^{\circ}$ . Where these are mentioned both counts are given, but unless explicitly stated heat units are computed only from  $10^{\circ}$ .

TABLE I  
TIME REQUIRED FOR THE DEVELOPMENT OF NEMATODES IN CULTURE-SOLUTION EXPERIMENTS, STARTING FROM APPEARANCE OF GALL

Temp., °C	To stage 8		To stage 9		To stage 10		To stage 12		To extrusion of ootheca		To oviposition			To hatching of eggs	
	Days	Nema- todes	Days	Nema- todes	Days	Nema- todes	Days	Nema- todes	Days	Nema- todes	Days	Nema- todes	Eggs laid per female	Days	Cultures
31.5			10	1	9*	1	15-17	2	10*-17	2	10*	1	19		
30.0							16-17	6	17	1	18-20	3	7-75	31-35	2
29.5			8	1	15	1			17	1	17	4	1-7		
29.0					12-16	2									
26.0															
25.0															
24.5	11	1	8-10	2	19	1	19-20	3	19-21	3	19-21	4	2-50	35	1
24.0	7-11	2	10-12	2	11*	1	16-20	5	19-21	5	19*-25	6	1-50	29*-39	4
23.5			7*	1	19	1	19-24	19	24	2	22-26	16	1-121	35	1
23.5							21-25	4	21-24	3	21*-27	7	8-236		
22.5							21	1			24*	1	28		
22.0			13	1											
18.5							42	1			42	1	5		
18.0							34*-45	4							
17.5									44		44*-47	5	1-20	79*	1
17.0															
16.5							50-54	7	52-53	2	50-58	13	1-65	83	1
16.0							56	2	56	1	53*-56	4	1-30	84*	1
15.5											59-63	2	1-40		
15.0							69-70	3	68	1	64-68	4	1-9		
15.0							68-73	4			69*-76	6	1-30		
14.5					46*	1					75*-80	7	1-40		
14.0					54*	1									
13.3							103	1			96-104	2			
13.0									91*	1					
12.5															
12.0	49	1	53	2											
12.0	26*	1	46*-103	7	69*-110	9									
11.5	49*-100	6	82-97	8	82*-110	9									
11.0	78-83	2	84	1											
10.7	109-161	6	110-157	2	151*	1									
10.0	92-162	10	99-148	2											
9.5	149-163	3	163*	1											

\* Record of minimum time at this temperature plotted in figure 2. In 3 cases, records of egg-laying at two temperatures a quarter of a degree apart, not shown separately in the table, are both included in figure 2.

† In the 28-day culture, plotted in figure 2, larvae were fully developed but not hatched from the eggs.

## RATE OF DEVELOPMENT AT DIFFERENT TEMPERATURES

Tables 1 and 2 present the experimental data in condensed form. A wide range in rate of development is included, but the very slowest cases have been discounted and their records omitted.

From these data, examples of the most rapid rate of development for each stage have been selected for figure 2, a graph which shows the time of development at various temperatures and the relation of the different stages to the parabolas. The culture-solution experiments were started after gall formation, while the soil experiments were started with free-living larvae. In the graph the former records have been given their proper positions in relation to the minimum time required for gall formation, represented by the parabola of 500 heat units, so that the stages of one complete life history can be pieced together from the several observations.

TABLE 2

TIME OF DEVELOPMENT OF NEMATODES IN SOIL EXPERIMENTS, STARTING WITH FREE-LIVING LARVAE

Temp., °C.	To stage 8		To stage 9		To stage 10		To stage 12		To oviposition		
	Days	Nema- todes	Days	Nema- todes	Days	Nema- todes	Days	Nema- todes	Days	Nema- todes	Eggs laid per female
35.5	7*-8	2	10	1							
34.0	10	1	12	1	12*	1					
33.5			8*	3	14	3					
31.5	10	1	7*-13	3	13	2	14*-17	6	17*-22	5	1-25
30.0	7	1	10	1	13	3	13-16	7	17-18	2	17-28
28.5	7-10	11	7*-10	13	12-14	34	13-18	53	17	7	1-50
28.0			10	1	10*	1	13*-17	64	17	3	1
27.5	7-10	21	7-10	10	15	3	17	44	17	4	1-5
27.0	7-10	9	7-10	5	12-15	6	15-17	59	16*-19	5	1-15
26.5	8	4	10-12	2	14	3	17-19	11	20-21	38	1-150
26.0	6-10	3	7*-12	4	13-17	6	19	1	21	75	2-150
25.5	11	1	11	1	14-16	9	16-20	37	20*-22	26	1-50
25.0	6-10	13	8-13	4	13-16	4	16*-20	4			
24.5	7	1	14	1	17-21	9	20-22	58	21-30	10	3-80
24.0	6*-12	57	9-15	14	14-20	34	18-20	3	25-27	25	15-150
23.5	9	8			19-21	9	19*-24	24	28-29	9	25-100
23.0	9-13	14	13-16	4							
22.5	11-12	4	11-16	43	17-18	14	25-26	35	26	5	1-20
20.0	11	1	14*	1	25	1	26*-31	2	31*	1	15
19.0	17-20	18	17*-20	13	26-29	5	38	4			
15.5									67	2	5-35
14.0	44	1									
13.5					56*	4	73*	2			
12.8	41	1									
12.2	50	2									

\* Record of minimum time at this temperature plotted in figure 2.

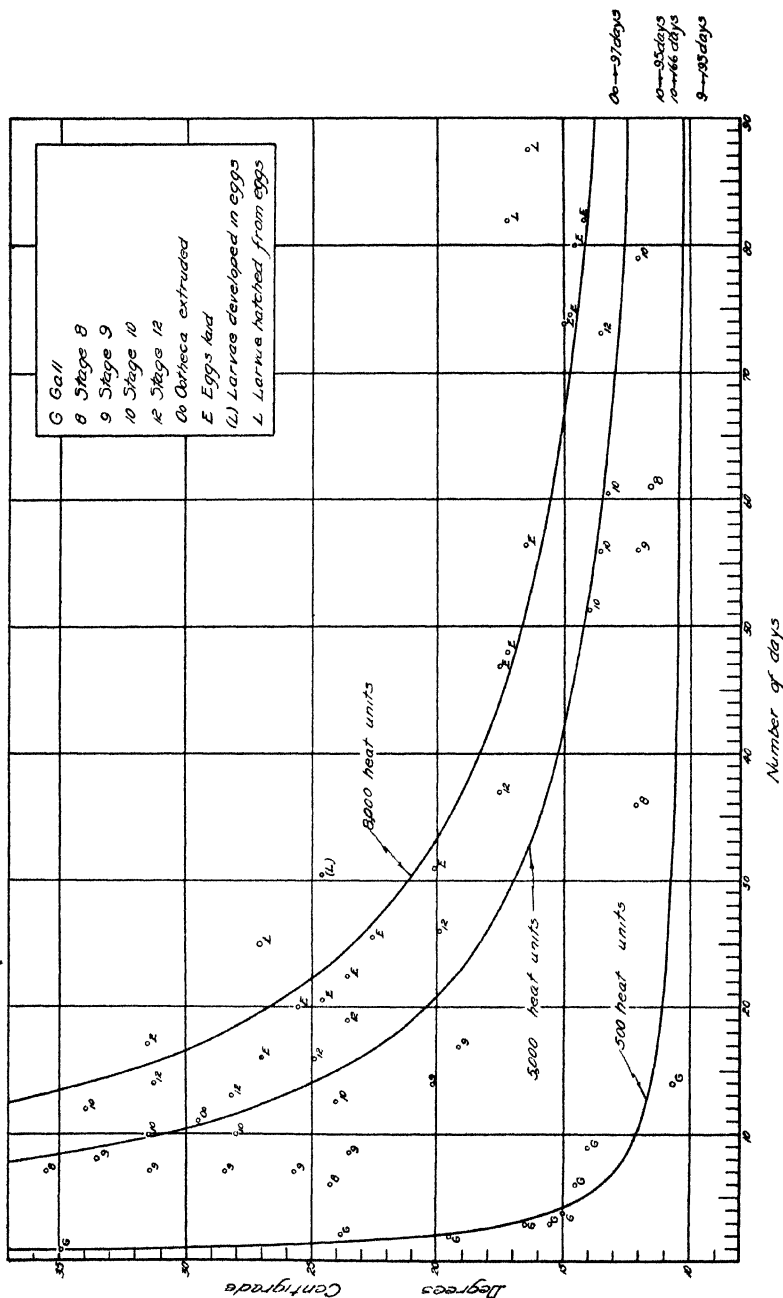


Fig. 2. Minimum time at various temperatures for the development of nematodes from free-living larvae through several stages of growth to the extrusion and hatching of eggs. The curve of 500 heat units above 10° C represents the minimum time allowance for gall formation. The other unit curves are reference lines only.

This graph (fig. 2) shows that the minimum time required for the life cycle in tomato roots from free larva to free larva was 25 days at 27.0° C, increasing to 87 days at 16.5°. The minimum time from the appearance of a gall to the beginning of egg-laying was 15 days at 27.0° and nearly 79 days at 14.3°. In the medial range the rate of increase of time with decreasing temperatures was fairly steady and regular.

TABLE 3

VARIATION IN AMOUNT OF DEVELOPMENT OF NEMATODES UNDER LIKE CONDITIONS

Temperature, °C	Days	Heat units	Root growth	Number of cultures	Development of nematodes
17.0	53	8,508	Fair	{ 1 1 2	Stage 10 Female with oötheca Females containing full-grown ova
24.0	25	8,357	Fair	{ 1 2 1 1 1	Female containing ova 6 eggs laid 34 eggs laid 110 eggs laid 121 eggs laid
24.0	24	8,018	Good	{ 1 2 2 1	Stage 10 Stage 12 Females with oöthecae 20 eggs laid
24.5	19	6,746	Good	{ 1 3 1	Stage 10 Females containing ova 2 eggs laid
29.0	16	7,232	Fair	{ 1 1 1	Stage 9 Stage 10 Female containing ova

The rate of development is dependent to an appreciable extent on the factor of nutrition, which cannot be controlled without a method of completely artificial cultivation. Even at moderate temperatures there was considerable variation in rate or in amount of development in Pfeffer's solution cultures that were identical in all external conditions, including a similar amount of root growth. The data for several groups of cultures compared in this way are shown in table 3. For each group, the galls were formed on the same day by offspring of one unmated female, and were incubated side by side. The records for 24.5° C show that it was possible under the conditions of that experiment for one female to lay 2 eggs and for three others to contain partly developed ova, while a fourth individual had reached only stage 10. The slower individuals must also be considered, of course, and the average rate of development will be presented later, in connection with figure 3. However, the minimum period of development, as shown in figure 2 by the more advanced

individuals at the various temperatures, is of the greatest practical significance because it is experimentally the most accurate, and because of its application in control operations by the fallow-field and trap-crop methods. The differences in rate of development among cultures in the same host species and the differences found by Godfrey and Oliveira (1932) in different hosts may perhaps be interpreted as different manifestations of the fundamental relation between nutrition and rate of development.

## STATISTICAL PRESENTATION OF RATE OF DEVELOPMENT

Figure 3 is a mean-velocity curve based on the data for egg-laying presented in tables 1 and 2. Each line represents a group of records averaged by the method of least squares. Temperature and rate, the reciprocal of the time of development, are the variable factors compared.

Before they were averaged, the figures were corrected in such a way that all records should cover only the time from the appearance of a gall to the first extrusion of eggs. The minimum time required for gall formation (as shown in figure 2 by the curve of 500 heat units) was therefore subtracted from the recorded time of the soil experiments; and where a female with an egg mass was found in any experiment, the recorded time of her life history was corrected by the allowance of 12 units for each egg laid, which will be explained later.

In spite of the irregularities indicated in table 3, a correlation coefficient of 0.97 was obtained for the 279 records from  $14.3^{\circ}$  to  $28.0^{\circ}$  C. To determine the " $\alpha$  point" (Shelford, 1927), the curve for these records has been extrapolated to the temperature axis, which it cuts at  $11.5^{\circ}$ .

At temperatures under  $16.5^{\circ}$  C, all the records fall below the first curve. Therefore a second curve, with an  $\alpha$  value of  $10.2^{\circ}$ , has been added to the graph, showing the relatively more rapid rate of development at low temperatures. Although the second curve was made from only 10 records, between  $14.3^{\circ}$  and  $14.8^{\circ}$ , its direction is almost identical with one made from the 22 records from  $14.3^{\circ}$  to  $16.3^{\circ}$ .

The curve for the 22 records above  $28^{\circ}$  C, averaged separately, shows the reversal of direction which indicates that the average rate is retarded at high temperatures.

The same types of deviation from the straight line, beyond the temperature limits of normal development, have been found by other investigators (Glenn, 1922; Peairs, 1927; Shelford, 1927), following the observations of Krogh (1914). Shelford (1927) uses the term "medial temperatures" in referring to the temperature range within which the data from experimentation coincide with the parabola of time and temperature or with its reciprocal, the straight line of rate and temperature.

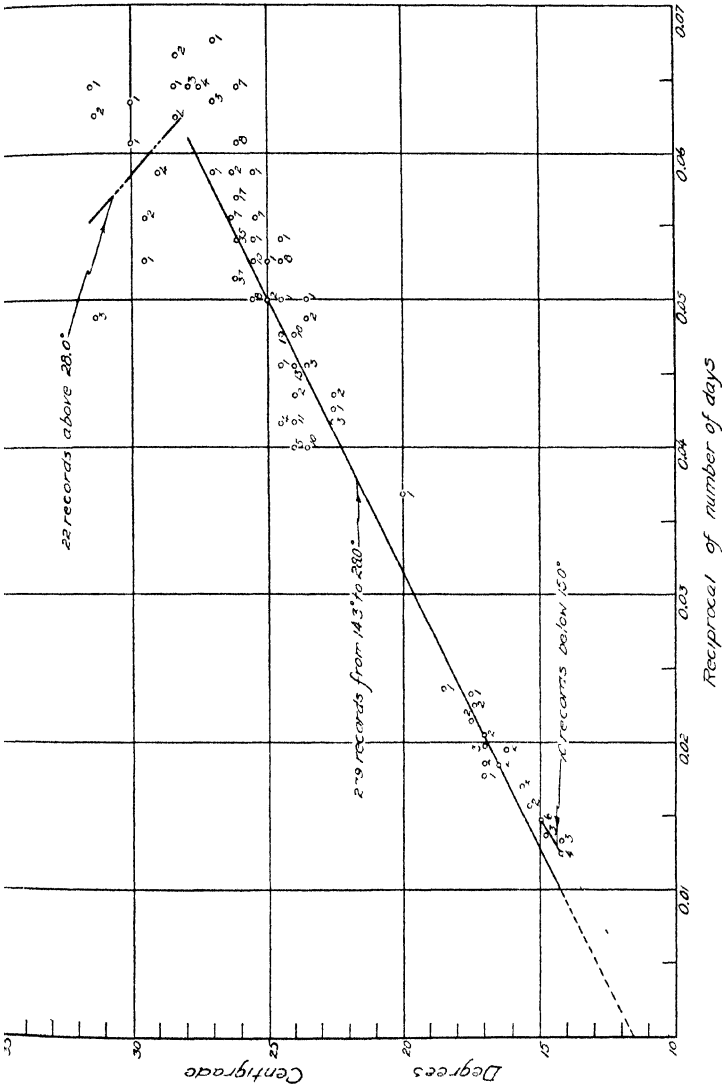


Fig. 3. Rate of development of nematodes from gall formation to egg laying: an average of 279 records from 14.3° to 28.6° C. This curve has been extrapolated by a dotted line which shows the  $\alpha$  point at 11.5° where the line cuts the temperature axis. The 10 records below 15° were averaged again, separately, to form the basis for the lower line with its  $\alpha$  point at 10.2°, showing the relatively more rapid rate of development at low temperatures. The 22 records from 28.3° to 31.5° form the upper curve, which shows the rate of retardation at high temperatures. The distribution of the experimental data is indicated by numbers representing nematodes.



## THRESHOLD OF DEVELOPMENT

An experiment was made to test the lowest temperature possible for nematode development, using newly formed galls in Pfeffer's-solution cultures. There was no development in 33 cultures kept between 7.0° and 7.5° C for 50 to 105 days, but in similar cultures stored at this temperature for 58 to 77 days and then removed to room temperature, 5 of the 14 nematodes developed and laid viable eggs.

At 9.5° C there was development as far as stage 9, so the threshold temperature must be at or below 9°. The roots also made some growth.

It is possible that the threshold is higher for maturity and egg formation than for the early stages of development. The velocity curve for the entire life cycle has its a point at 11.5° C (fig. 3), and also the egg-laying records in figure 2 would be more harmonious with parabolas calculated from 11.0°, while stages 10 and 12 are harmonious with the parabolas represented on the graph, which were calculated from 10.0°. Ludwig (1928), working with the Japanese beetle, and Glenn (1922), with the codling moth, found that the egg, larva, and pupa each showed independent threshold and maximum temperatures.

## DEVELOPMENT AT LOW TEMPERATURES

Eggs were laid at 14.3° C, but the greatest development accomplished at a constant lower temperature was the extrusion of the oötheca at 13.3°. This occurred in 3 cultures out of the 13 which had fair root growth and sufficient time allowance. Yet at still lower temperatures, not held constant throughout the experiment, 2 females contained partly developed ova: one culture had been kept for 297 days at several steady temperatures between 7.0° and 12.5°, with a total of 7,722 heat units above 10.0° or 12,642 above 9.0°; the other had been 255 days between 7.0° and 12.5°, plus 9 days at 14.0°, with 6,569 or 10,721 heat units. The failure at 13.3° can probably be explained as the result of inadequate nutrition, but the possible stimulating effects of temperature changes<sup>3</sup> and of subthreshold temperatures (Parker, 1930) should be

<sup>3</sup> Peairs (1927) considers that constant temperatures are unnatural, and may tend to retard development. Ludwig (1928), on the other hand, found that the Japanese beetle developed as rapidly at constant as at fluctuating temperatures, when the effects of extreme temperatures were eliminated and fluctuation took place only between the threshold and the optimum temperature. However, Cook (1927), Peairs (1927) and Parker (1930) have all found an accelerated rate of development for the insects investigated, correlated with daily fluctuations between the threshold and the optimum temperature. The nematode cultures under discussion are not exactly comparable with the experiments quoted from other authors, because the nematodes were exposed to small daily fluctuations of temperature during only a part of the period of development, and for the remaining time the temperature changes occurred at intervals of several weeks.

borne in mind (in connection with the 2 cases where ova were formed at even lower temperatures). However, in the 10 additional cultures at irregular temperatures below 12.5° which had sufficient time and root growth, only one nematode had reached even the oöthecal stage. These cases with irregular temperature records are not included in the tables or graphs, which are based exclusively on experiments at constant temperatures.

At still lower temperatures, maintained more or less constant, there were 334 cultures started at 10°, 11° and 12° C, not all of them reported in the tables. They were examined after several months in cold storage, although their heat-unit counts were still low. Of 320 cultures with less than 4,000 heat units, 90 showed more development than would have been expected from their low unit counts, 95 showed an average rate, and 135 were either very slow or else arrested before examination. Stage 10 was reached 16 times, once with as few as 1,475 heat units above 10°, or 3,400 above 9°, the other 15 times with 2,400 to 3,600 heat units above 10°. The 14 cultures with longer growth time, which had accumulated from 4,000 to 5,200 units above 10°, showed no development beyond stage 10.

Peairs (1927) points out that there is "an apparent loss of vitality incident to the prolonged vital period" at low temperatures. This applies both to host plant and to parasite. Nematodes probably have higher nutritional requirements for maturity and egg formation than for the early stages, while the tomato seedlings failed to continue their growth through the time required for nematode development at these low temperatures. No attempt was made to substitute some species of plant which would make a relatively better growth at low temperatures, because of the host differences found by Godfrey and Oliveira (1932), but such an experiment might give illuminating information.

TABLE 4  
GALL FORMATION IN SOIL AT LOW TEMPERATURES

Temperature, ° C		Days	Hours above 13° C	Nematodes	
Range	Average			Number	Development
9-12.2	10.6	14*	0	1	Stage 3
10.4-13.3	12.0	43	42	1	Stage 7
10.4-13.7	12.2	56	171	2	Stage 7
10.4-13.7	12.2	50	171	2	Stage 8

\* This record is plotted in figure 2.

## ROOT PENETRATION AT LOW TEMPERATURES

Root penetration requires considerable activity on the part of a larva, and its occurrence at very low temperatures was not anticipated. Godfrey (1926) considered 13° C as the critical temperature for gall formation in roots growing in soil. A few galls found at lower temperatures in my 1927 experiments in soil are reported in table 4. In only one case was the soil temperature below 13° during the entire time of the experiment, but the others averaged around 12°. The first record is the only one in soil which shows the time required for gall formation, because the other experiments included a period of larval development within the roots. Experiments on gall formation in petri dishes (discussed in connection with table 10) were not made below 14°.

## DEVELOPMENT AT HIGH TEMPERATURES

While galls were formed and development was started at temperatures as high as 35.0° C, both in soil and in Pfeffer's-solution cultures, complete development was not obtained above 31.5°. In the 22 cases of egg-laying between 28.3° and 31.5°, a few individuals showed the same rate as at 27.0° (fig. 3); but there was no acceleration in response to the higher temperature, and the majority showed a marked retardation.

The curve for rate of development (fig. 3) breaks sharply above 28° C, showing that this is the optimum temperature, above which development is slower. The irregularity of distribution of the records above this temperature is an indication that the limits of normal development have been passed, although the factor of inadequate nutrition again enters at about 31°. Godfrey (1926) states that tomato roots show maximum gall formation at 30°, and that other roots vary somewhat according to the temperature of their optimum growth.

In culture-solution experiments at 36.5° C, the roots made little or no growth and all their growing tips were killed. In 14 to 24 days, 22 nematodes made no growth at all; 12 larvae fed and started to fatten but failed to reach stage 8; and only 1 grew farther, a male, which died before becoming fully developed. This observation is of interest in connection with the discussion of sex ratios in the foregoing paper (Tyler, 1933), but it seems less significant in the temperature study.

After 9 to 16 days at 36.5° C, 7 cultures (table 5) were given time to recover at room temperature. Although the galls were dissected with great care none of the 7 nematodes was found, and it was concluded that

TABLE 5  
TESTS OF RECOVERY OF NEMATODES FROM EXPOSURE TO HIGH TEMPERATURES

Number of cultures	Initial treatment			Subsequent incubation		Total root growth	Nematodes	
	Temperature, °C	Days	Possible development of nematodes*	Temperature	Days		Total development	Recovery
1	36.5	6	Stage 9	25° C	33	None	Stage 12	Partial
1		9	Stage 10	24° C	30	Poor	Nematode not found	None
3		14	Formation of ova	Room	51	Poor	Nematodes not found	None
2		15	Egg-laying	Room	{ 20 51	None	Nematode not found	None
1		16	Egg-laying	Room	51	Fair	Nematode not found	None
2	37.0-39.0†	4	Stage 7	24° C	20	Fair	{ Stage 3 Extrusion of oötheca	None Partial
1	37.0-40.0‡	14	Stage 5	{ 31° C 25° C	{ 32 22	Fair	Female containing ova	Partial
2		2	Stage 5	31° C	32	Fair	Stage 12	Partial
1		3	Stage 6	31° C	12	Fair	Stage 7	Slight
1		5	Stage 8	31° C	12	Poor	Stage 8	None
2		6	Stage 9	31° C	12	Poor	Stage 3	None
3		8	Stage 10	31° C	12	Fair	Stage 3	None

\* Possible development of nematodes was determined from the data of figure 2, on the basis of time.

† Only 13 hours above 38.0°.

‡ Only 10 hours above 39.0°.

there was no development but probably death in the larval stage. The roots were stunted by the high temperature, and only 2 of them made new growth during the period allowed for recovery at room temperature. In 13 other cultures (table 5) exposed for shorter periods at 36.5° to 39.0°, 5 nematodes made some recovery, but only 1 female formed ova and these were only partially developed.

The records are clear on the subject of recovery, although the nematodes were not examined until the end of the experiment. Their possible development during the time of the initial treatment was tabulated by analogy with the cultures (fig. 2) which showed the greatest development for the given number of days, although as a matter of fact, development is retarded at such a high temperature. If at the end of the experiment a nematode showed more development than was possible during the initial treatment, recovery was recorded. Three of the 5 nematodes showing recovery had suffered only the shortest exposures at the high temperature, and only 1 of the 5 had a theoretically possible development during that treatment beyond stage 8, while the nematodes which made no development at all were those which had suffered the longest exposures to the high temperature.

The irregular results shown in table 5 are to be expected at such an extreme temperature. On the evidence of the 55 complete or partial failures in culture-solution experiments, it is concluded that the maximum temperature for the development of the root-knot nematode in such cultures is around 36.5° C.

Another culture-solution experiment (table 6) showed that an exposure of 5 days at 36.5° C did not kill nematodes which were already in more or less advanced stages of development. Young females continued to form viable eggs when returned to a medial temperature after the exposure. There was some unhealthy development after a 7-days' exposure at 36.5°. Possible development during the initial incubation at 25.0° was calculated by analogy with other cultures at medial temperatures on the basis of heat-unit counts.

It is barely possible that certain resistant individuals might develop at slightly higher temperatures in healthier roots. Indeed, in a preliminary experiment in 1925, 2 females reached stage 12 at temperatures around 40° C. The host plants were growing in jars of infested soil, with their tops in the cooler air of the room. The thermostatic control was accurate, and the other data from this preliminary experiment were similar to those in the later experiments; but because there was no arrangement for circulation of the heated water around the jars, none of the records are tabulated here.

TABLE 6  
EFFECTS OF HIGH TEMPERATURE ON PARTIALLY DEVELOPED NEMATODES

Number of cultures	Initial incubation at 25.0° C			Subsequent treatment				Nematodes	
	Days	Heat units	Possible development of nematodes*	Root growth	At 36.5° C		At 25.0° C		Recovery
					Days	Heat units	Days	Heat units	
1	3	1,015	Stage 5	Healthy	3	1,892	37	13,381	Fair
1	10	3,612	Stage 9	Good	3	1,892	37	13,381	Fair
2	18	6,453	Extrusion of oötheca	Fair	3	1,892	26	9,328	Good
1	3	1,055	Stage 5	Fair	5	3,171	35	12,677	None
2	17	6,089	Stage 12	Good	5	3,171	35	12,677	Fair
1	19	6,823	Early egg formation	Fair	5	3,171	35	12,677	Good
1	2	721	Stage 5	Good	7	4,553	24	8,625	Good
1	10	3,612	Stage 9	Poor	7	4,553	33	11,976	Fair
1	11	3,975	Stage 9	Fair	7	4,553	33	11,976	Fair
1	18	6,453	Extrusion of oötheca	Poor	7	4,553	33	11,976	Fair
1				Good	7	4,553	33	11,976	None
									None

\* Possible development of nematodes was determined by comparison with other cultures at medial temperatures on the basis of heat-unit counts.

The limit of survival for many animals is around 40° C. Other experiments with nematodes at high temperatures have used only the free-living stages, so that it is not certain whether the high temperature was directly lethal, or whether death was caused by exhaustion of food reserves, as Baunacke (1922) suggested. Frandsen (1916) found that at 40° development of eggs of the root-knot nematode was accelerated for a short time, and that some eggs hatched before the embryos were completely developed, but that after 18 hours at this temperature all eggs and larvae were dead. Hoshino and Godfrey (1933) report that larvae of this species were killed in water at 40° in 2 hours and 15 minutes. Baunacke (1922) found that larvae of the sugar-beet nematode became rapidly exhausted at 29° and above, but that motion continued feebly in some individuals up to 37.5° and even to 39° if heated very gradually. Although all larvae were quiescent at 40°, most of them were able to recover slowly from a short exposure at that temperature.

The fact that the optimum temperature is found so far below the maximum is probably related to the temperature conditions most often occurring in nematode-inhabited soils.

#### ROOT PENETRATION AT HIGH TEMPERATURES

Root-penetrating activity of larvae continued at high temperatures, though the results were somewhat irregular. In the 1927 experiments with plants growing in soil, there were 26 roots at 33.0° to 35.0° C without galls, and only 6 roots attacked, in which 8 nematodes were found at stages 5, 6, and 7, and 17 at stages 8, 9, and 10, though they had all had time for further development. There was also a male at stage 14. One gall was found on a root which had been 42 hours in infested soil above 40.5°. From the preliminary experiments of 1925 there are records of 4 galls formed at 40.0°, containing nematodes at stages 8 and 9.

Most of the galls formed in petri-dish cultures at 35° C appeared after 1 or 2 days of incubation (table 10). After this galls were not formed at the high temperature, but some of the larvae survived a 5 days' exposure and were again capable of active root penetration when the plates were removed to a medial temperature. Later development of the nematodes was not affected by the conditions of this experiment.

#### COMPUTATION OF RATE OF EGG-LAYING

The rate of egg-laying was reported by Bessey (1911) as 10 to 15 or more eggs a day, without reference to the temperature, and by Byars (1914) as one egg an hour, or one in 50 minutes under the heat of a strong microscope light.

In calculating the rate of egg-laying in my experiments, two methods were used. The first was a trial-and-error method. The number of heat units required for development to the beginning of egg-laying was calculated for 301 females (fig. 3), most of which had laid more than one egg. It was found that an allowance of 12 heat units per egg gave the most consistent results.

The second method, used to check the first, was based on a comparison of pairs of records. If two females cultivated at the same temperature developed at the same rate, they would reach the stage of egg-laying

TABLE 7

VARIATION IN TIME REQUIRED FOR DEVELOPMENT OF NEMATODES FROM GALL FORMATION TO EGG-LAYING IN CULTURE-SOLUTION EXPERIMENTS

Temperature, ° C	Days	Heat units	Number of cultures	Eggs laid
25 0	20	7,017	1*	2
24 5	19	6,562	1†	5
24 5	19	6,746	1	2
24 0	24	8,018	2	7
24 0	25	8,357	2	6†
24 0	25	8,125	1	8
23 5	21	6,698	2	8

\* Culture B of table 8.

† Culture A of table 8.

Under identical conditions, other nematodes laid from 34 to 121 eggs (see table 3).

with the same number of heat units. On this assumption, if one culture is examined at the beginning of egg-laying and the other is allowed a longer period of development, the difference in number of heat units between the two cultures would correspond to the difference between the two egg counts, and indicate the heat requirements for laying that number of eggs.

However, the rate of development is not the same for all females. Table 3 shows the wide variation in amount of development for the same time and temperature, and table 7 shows the range of time, measured in heat units, required by different nematodes at approximately the same temperature for the same amount of development. Table 3 includes some of the records of very slow development which were omitted from table 1.

Since it would obviously be inaccurate to compare two females for rate of egg-laying unless their rates of development were practically the same for the entire life history, it is necessary to interpret the differences. Simple inspection of the data in tables 1 and 2 shows which cul-



tures made rapid and which slow development. Of the 10 records given in table 7, the culture showing the shortest time to egg-laying is called A, and a somewhat slower culture, B, was selected as having a rate more representative of the group. The 13 "longer-time" cultures at the same temperature, which had from 15 to 390 eggs apiece, were compared with A and with B, as illustrated for 2 of them in table 8. The first of these, No. 4910, averaged 10.7 units per egg when compared with A, and 9.4

TABLE 8

METHOD OF CALCULATING THE NUMBER OF HEAT UNITS FOR EGG LAYING

	Comparison with short-time culture A		Comparison with short-time culture B	
	Eggs laid	Heat units	Eggs laid	Heat units
Longer-time culture No. 4910	390	10,687	390	10,687
Short-time culture	5	6,562	2	7,017
Difference	385	4,125	388	3,670
Heat units per egg		10.7		9.4
Longer-time culture No. 5798	15	7,178	15	7,178
Short-time culture	5	6,562	2	7,017
Difference	10	616	13	161
Heat units per egg		61.6		12.4

when compared with B, indicating that the entire rate of development of this culture was more rapid than that of B, and even perhaps of A. In the second comparison shown in table 8, culture No. 5798 approached the rate of B, as shown by the average of 12.4 heat units per egg, as against 61.6 units when compared with A. The small number of eggs makes the difference in average number of units per egg more striking, but the retardation indicated by this high average probably occurred throughout development, and should not be referred entirely to the period of egg laying.

Similar comparisons were made with pairs of cultures at 29° C. In most cases at both temperatures, one of the comparisons gave a result between 10 and 20 units per egg, though there were two instances of development so slow that both comparisons gave absurdly high results. It was concluded from this calculation also that 12 units per egg is a reasonable allowance. This would be 1 egg an hour at 22°, or 12 eggs a day at 16°.

## RATE OF DEVELOPMENT OF EGGS

To give information on the complete cycle, the rate of embryonic development was investigated. Recently deposited egg masses from culture-solution experiments were returned to the incubators and watched for larvae. Another and more exact method was to observe the development of individual eggs in distilled water or saline solutions, sealed in hanging-drop cultures. Table 9 reports both of these experiments. There is individual variation, as in all biological material, and the data are less

TABLE 9  
TIME REQUIRED FOR DEVELOPMENT AND HATCHING  
OF EGGS IN VITRO

Temperature, ° C	Days	Eggs
29.5 .....	9-11	16
27.0 .....	9*-11	22
26.5 .....	12-13	21
25.5 .....	12-15	14
24.5 .....	15-17	10
Room .....	19-25	9
17.5 .....	26	1
17.0 .....	28-31	17
16.5 .....	31*	7

\* Record of minimum time at this temperature plotted in figure 2.

extensive than those on other stages of the life cycle, but they give a consistent curve (fig. 2). Eggs hatched after 9 days at 27.0 C and after 31 days at 16.5°, counting from the 1 or 2-celled stage.

Godfrey and Oliveira (1932) report hatching of eggs in 5 days, at a temperature around the optimum, in galls on cowpea. This is a much shorter time than is found in any of my records, but since these authors allow 19 days before the discovery of the egg mass, the length of the entire cycle, 24 days in their experiment, is only one day less than my most rapid case.

## RATE OF ROOT PENETRATION

The length of time required for gall formation at different temperatures is shown in table 10 and figure 2. As soon as plate cultures were started with one active larva beside a seedling in a petri dish (for method see Tyler, 1933), the plates were placed in an incubator chamber and examined for galls at 12-hour intervals. Galls were formed in plates at

15° C in a minimum of 4 days. The rate increased at higher temperatures, and at 35° macroscopic galls were found within 21 hours. That actual entrance into the root probably occurred sooner than this is indicated by the stained preparations of Godfrey and Oliveira (1932), which demonstrate larvae inside the root tips of cowpea and pineapple

TABLE 10  
TIME REQUIRED FOR GALL FORMATION IN TOMATO  
SEEDLINGS BY NEMATODE LARVAE IN  
PETRI DISH EXPERIMENTS\*

Temperature, °C	Days	Number of larvae penetrating roots
35.0	0.8†-1.0	5
	1.5-2.5	22
	3.5	1
23.8	2.0†-7.0	8
19.5	2.0†	1
	3.0-6.0	12
16.5	3.0†-4.0	7
	5.0-11.0	9
16.0	6.0-7.0	2
15.5	3.0†	1
15.0	4.0†-6.0	6
	11.0-14.0	3
14.5	6.0†	1
14.0	9.0†-11.0	2

\* There is also a record of gall formation in soil in 42 hours or less at 40.5° to 44.0° C and another in 14 days at 9.0° to 12.2°

† Record of minimum time at this temperature plotted in figure 2

6 hours after inoculation, although galls did not appear until nearly 2 days had elapsed. The discrepancy between the time of root penetration and the appearance of the gall was not so great in my plate cultures. The root tip became opaque while the nematode was still only partly buried in its tissue, and a definite swelling appeared a few hours later.

## SUMMARY

The minimum time required for the life cycle of the root-knot nematode from larva to larva in experiments in tomato roots was 25 days at 27.0° C, increasing to 87 days at 16.5°. Development from gall formation to egg-laying required 15 days at 27.0° and 79 days at 14.3°.

The velocity curve shows acceleration of rate of development with rising temperatures through the medial range, and marked retardation of the average rate above  $28.0^{\circ}\text{C}$ , although cases were found which showed practically the same rate at  $31.5^{\circ}$  as at  $27.0^{\circ}$ . Below  $16.5^{\circ}$  development was relatively more rapid than the average shown by the velocity curve. There are also considerable variations in rate of development for individual nematodes, possibly related to their nutrition.

As a working guide for all stages, heat units were computed as hour-degrees above  $10^{\circ}\text{C}$ . Development to egg-laying required from 6,500 to 8,000 such units.

Root penetration by larvae in soil occurred at temperatures as low as  $12.0^{\circ}\text{C}$ . Early development occurred as low as  $9.5^{\circ}$  in cultures, indicating that the threshold of development is around  $9^{\circ}$ . After newly formed galls had been stored for 77 days at a temperature of  $7.0^{\circ}$ , a few of the nematodes were still able to develop normally at room temperature. No eggs were laid below  $14.3^{\circ}$ , but whether this limitation was related to temperature or to nutrition was not determined. The threshold temperature for maturity and egg-laying appears to be higher than for the early stages of the life cycle.

Serious injury to developing individuals in cultures was found at  $36.5^{\circ}\text{C}$ , possibly correlated with the condition of the host plant. Free larvae, however, were still capable of root penetration after 5 days in plates at  $35.0^{\circ}$ , and one gall was found in soil above  $40.5^{\circ}$ . Eggs were not laid above  $31.5^{\circ}$ .

The rate of egg-laying was computed roughly as one egg per hour at  $22.0^{\circ}\text{C}$ , or 12 units above  $10.0^{\circ}$  for each egg laid.

Eggs developed from the 1 or 2-celled stage to hatching in 9 days at  $27.0^{\circ}\text{C}$  and in 31 days at  $16.5^{\circ}$ .

Root penetration and gall formation in petri-dish cultures required 4 days or more at  $15.0^{\circ}\text{C}$ , decreasing to 21 hours at  $35.0^{\circ}$ .

## ACKNOWLEDGMENTS

Grateful acknowledgment is made to Professor W. B. Herms for ecological interpretations as well as for counsel on the manuscript, and to Dr. W. M. Hoskins for assistance with apparatus and with analysis of data.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

APRIL, 1933

No. 11

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# A COMPARISON OF AONIDIELLA AURANTII AND AONIDIELLA CITRINA, INCLUDING A STUDY OF THE INTERNAL ANATOMY OF THE LATTER<sup>1, 2, 3</sup>

ROBERT G. NEL<sup>4</sup>

This investigation was undertaken for two reasons—first, to determine the actual status of *Aonidiella aurantii* (Mask.) and its so-called variety, *A. citrina* (Coq.) ; and second, to learn more about the internal anatomy of the Diaspidinae, as certain structural features still seem to be matters of controversy.

Because of their distinct differences in color and mode of attack, *aurantii* and *citrina* are respectively recognized as the red scale and yellow scale. In fact, so specific are they in these differences that their identity is hardly ever confused ; to workers in the field the names red and yellow scales mean two different insects. From a systematic viewpoint, however, distinction has not been so clear-cut, as no morphological differences could be detected upon which a differentiation could be based, with the result that *citrina*, instead of being given specific standing, has been classified as a variety of *aurantii*.

## HISTORY AND SYNONYMY

By referring to Fernald's<sup>(8)</sup> *A Catalogue of the Coccidae of the World*, and to subsequent publications on the Coccidae, one finds that *Aonidiella aurantii* (Mask.) has been described under many different names. As a result, a long list of synonyms has gradually been built up. It is not within the scope of this paper to enter into an extended discussion of these synonyms. The writer will, therefore, limit himself only to those main genera that played the most important roles in the complicated systematic history of the species concerned.

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<sup>1</sup> Received for publication June 18, 1932.

<sup>2</sup> Paper No. 238, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California

<sup>3</sup> Study begun and carried on for a year at the Citrus Experiment Station, Riverside, and completed at Cornell University as a thesis presented to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1930.

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*Aonidiella aurantii* (Mask.) belongs to the subfamily Diaspidinae, which is generally regarded as probably the best known of the Coccidae. This species was first described by Maskell<sup>(22)</sup> in 1878, under the genus *Aspidiotus*, and soon after by Comstock,<sup>(5)</sup> as *Aspidiotus citri*. The latter author, however, upon exchanging specimens with Maskell, immediately came to the conclusion that the two were identical. In 1895, Berlese and Leonardi<sup>(11)</sup> erected a new genus *Aonidiella*, taking *aurantii* as its type. In 1899, in the supplement of his *Check-List of the Coccidae*, Cockerell<sup>(4)</sup> placed *aurantii* under *Chrysomphalus*, giving no reference for this change. Since then, especially in the field of economic entomology, workers throughout the world have given *Chrysomphalus* preference as the genus for *aurantii*. However, the Italian school has consistently used *Aonidiella*. In 1921, MacGillivray,<sup>(20)</sup> in his classification of the Coccidae, also listed *aurantii* under *Aonidiella*, which he recognized as a valid genus.

Specimens of *Aspidiotus hederae* (Vallot) and *Chrysomphalus aonidum* (Linn.), being type species of respective genera, were carefully studied by the writer in order to obtain a better understanding of the generic characters in question. The original and subsequent descriptions were also taken into consideration.

As a result of these studies, the writer feels convinced that not only is *Aonidiella* a valid genus, but that *aurantii* is more properly classified here than under either *Aspidiotus* or *Chrysomphalus*. In fact, the types of *Aspidiotus* and *Chrysomphalus* show a closer relationship to each other than does *aurantii* to *aonidum*. For example, in both *Aspidiotus hederae* and *Chrysomphalus aonidum*, circumgenital glands are present, while both possess bodies generally turbinate in form; circumgenital glands, on the other hand, are wanting in *Aonidiella aurantii*, while its body is typically reniform in shape, as a result of the posterior elongation of the lateral thoracic lobes.

However, in order to determine the exact position of these species and the validity of the above-named genera, a detailed study of the Diaspine group should be undertaken. Time did not permit such an investigation in conjunction with the work described in this paper, as it would take at least a few years to obtain the necessary types.

The first description of *Aonidiella citrina* (Coq.) was made by Coquillett<sup>(6)</sup> in 1891, who was conducting some spray experiments on scale insects in the San Gabriel Valley, California. He referred to it as follows:

"The orange tree experimented upon was infested with the yellow scale (*Aspidiotus citrinus*)."

Notwithstanding its briefness and inadequacy, this description is recorded as the original.

It seems, however, that Coquillett did describe it more fully as a distinct species, although this description was never published, for the reasons brought out in the following statement, made by Dr. L. O. Howard<sup>(13)</sup> in 1894:

The Yellow Scale is mentioned in some California publications as *Aspidiotus citrinus* Coq. and Mr. Craw<sup>5</sup> is of the opinion that it is a distinct species and was imported independently from Japan in 1872 into the San Gabriel Valley. The name *Aspidiotus citrinus* Coq., was sent Professor Riley with a MS description, but from his own careful study in California, and correspondence with Mr. Coquillett, Professor Riley concluded that the structural differences between the two forms are not constant and that *citrinus* can only be considered as a variety.

From then onward, it continued to be classified as a variety of *aurantii*, and thus underwent the same generic changes, i.e., from *Aspidiotus* to *Chrysomphalus* and to *Aonidiella*.

In the following pages the writer has presented the result of a detailed study conducted on both these insects, and has come to the conclusion that there are sufficient morphological as well as biological differences to warrant giving *citrina* specific standing.

## DISTRIBUTION AND ECOLOGY

The red scale is widely known in practically all the subtropical and tropical countries of the world, having been recorded from southern Europe, Syria, Greece, Turkey, Italy, Spain, India, Ceylon, Mauritius, Australia, New Zealand, China, Japan, South Africa, Rhodesia, southern and western United States, Samoa, Java, and the West Indies. The yellow scale is not so widely distributed, having been recorded only from Japan, California, and Texas. It is probable that as a result of its similarity to the red scale, it may have been mistaken for, and recorded as, the latter.

The red scale in California, as noted by Quayle,<sup>(28)</sup> is chiefly a citrus pest, so that its distribution is governed largely by that host plant. In the citrus area south of the Tehachapi Mountains, this scale occurs in the following counties: Santa Barbara, Ventura, Orange, Los Angeles, Riverside, San Bernardino, and San Diego. In conjunction with the distribution of the yellow scale the above author states:

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<sup>5</sup> Craw, Alexander. Beneficial insects. Fourth Bien. Rept. Calif. Bd. Hort. p. 97. 1894.

It is widely distributed over the citrus belt of southern California, often associated more or less with *aurantu*. In addition to its occurrence in the southern part of the State, it is also found on the citrus trees of the Sacramento Valley where it is the most important scale occurring on citrus trees. In the same section the typical *aurantu* is not known. In the citrus belt of the south the yellow scale occurs in various degrees of severity ranging from occasional scales scattered about on parts of the tree, to badly infested trees.

From its general distribution, the yellow scale apparently shows a preference for the more arid and warmer interior valleys of central California or better withstands lower temperatures, while the red occurs more abundantly in the southern citrus area. In the Redlands-Riverside section, the yellow scale is most abundant in those sheltered groves lying along the foothills.

Perhaps the most important differences between the red and yellow scales are their modes of attack on the host plant. The red scale is found on all parts of the tree, whereas the yellow is limited almost entirely to the leaves and the fruit, a fact that makes the latter a less serious pest.

### LIFE-HISTORY OBSERVATIONS

The data presented here were obtained from records of insects reared on young seedling and Valencia orange trees. The experiments were conducted in the lath house during the warmer months, and were transferred to the insectary with the approach of winter. Table 1 gives the mean minimum and the mean maximum temperatures for the period during which most of the life-history studies were made.

TABLE 1  
TEMPERATURES DURING PERIOD  
OF LIFE HISTORY WORK

Year	Month	Mean maximum degrees Fahrenheit	Mean minimum, degrees Fahrenheit
1928	July	98 04	58 80
	August	95 09	58 35
	September	96 66	51 20
	October	82 48	43 84
	November	74 28	39 83
	December	66 16	35 13
1929	January	63 10	34 06
	February	62 90	33 52
	March	70 83	39 16
	April	69 83	43 67

During the course of these studies, the observations on the habits and the metamorphosis of both the red and the yellow scales were always made on a comparative basis. These observations are fully recorded (tables 2 and 3) and the conditions were identical for both, unless otherwise indicated.

TABLE 2  
SUMMARIZED LIFE HISTORY OF AONIDIELLA AURANTII

Experiment No.	Free-moving larvae			Duration molting period, days		Duration of instars, days			Total time, in days, from free-moving stage to start of reproduction	Adults secured	
	Date transferred (1928)	Number transferred	Number settled in 24 hours	First	Second	First stage	Second stage	Third stage*		Male	Female
100	8/10	55	24	4	3	12	9	32	60	11	16
105	8/31	78	54	3	4	13	9	32	61	14	30
108	9/1	31	28	3	3	15	7	33	62	11	12
109	9/4	30	24	3	4	14	8	31	60	5	13
110	9/5	40	40	4	3	13	8	34	62	9	21
111	9/6	147	128	4	4	13	9	35	63	31	80
131	9/27	160	151	3	4	12	10	31	62	55	77
132	9/29	180	144	3	4	12	9	32	61	66	70
Average.....				3.38	3.63	13.00	8.63	32.50	60.26		

\* From second molt to beginning of reproduction.

TABLE 3  
SUMMARIZED LIFE HISTORY OF AONIDIELLA CITRINA

Experiment No.	Free-moving larvae			Duration molting period, days		Duration of instars, days			Total time, in days, from free-moving stage to start of reproduction	Adults secured	
	Date transferred (1928)	Number transferred	Number settled in 24 hours	First	Second	First stage	Second stage	Third stage*		Male	Female
105	8/31	60	54	3	2	11	8	38	64	19	14
112	8/14	70	53	3	4	13	11	35	66	30	18
114	8/23	22	17	4	3	14	9	36	66	8	5
115	8/23	16	11	3	5	15	8	35	66	3	3
117	9/1	27	19	2	4	14	9	34	63	14	3
119	9/5	16	13	4	5	13	9	37	68	6	6
121	9/26	48	26	3	3	13	11	36	65	10	15
123	8/27	137	119	3	4	12	8	34	61	51	44
Average.....				3.13	3.75	13.13	9.18	35.63	64.88		

\* From second molt to beginning of reproduction.

First-stage or free-moving larvae, generally known as "crawlers," were procured by placing heavily infested oranges or lemons in battery jars provided with cheesecloth tops. In order to hasten the production of the larvae, the jars were kept in a fairly warm place. After a few days, the females produced many young which were removed from time to time. With the aid of a field binocular microscope and a fine camel's hair brush, the crawlers were transferred one by one to the leaves of young potted trees. Only those larvae that moved away from where they were placed, were recorded and used for further observation. This precaution was taken for two reasons: In many cases it was noted that when a larva was so transferred as to be accidentally placed on its back, it was unable to right itself, and died. The possibility existed that when an inactive larva was transferred, it was already in the process of settling and had probably pierced the epidermis of the fruit. Such a larva seemed unable to repeat the process of piercing the leaf.

In order to facilitate observation and to prevent the larvae from settling all over the tree, the following measures were adopted: All leaves on which larvae were to be placed were wiped off so as to remove dust, excessive moisture, and gum exudations. The larvae were kept in restricted areas by the use of leaf cages. Each leaf cage consisted of an upper and a lower cardboard frame, both lined on the surfaces adjacent to that of the leaf, with black velvet. This prevented the escape of the larvae under the frame and guarded the leaf against chafing. The upper frame was covered with a celluloid "window," and the lower one left open so that the transpiration of the leaf would not be disturbed. After transferring the larvae to the upper surface of the leaf, the latter was enclosed between the two cardboard frames which were held in position by means of paper clips. Observations could then be made through the celluloid window. The other method consisted of the isolation of the individual leaves. Prior to the transference of the larvae, the leaves were isolated by placing narrow bands of paraffin wax coated with tree tanglefoot, around their petioles. The few larvae which were trapped by the tanglefoot were counted and deducted from the total number transferred.

Of the above methods, the latter is preferable since direct observation under the microscope can be made without disturbing the settled colony. When the leaf cages are used, the larvae tend to crawl between the leaf surface and the velvet, thus becoming completely hidden. Also, the leaves frequently drop off because of the weight of the cages.

*First Larval Stage.*—This stage will be discussed under two sub-headings: the active or free-moving stage, and the fixed or settled stage.

The females of both the red and the yellow scales are viviparous. After birth the larvae remain a day or two under the protection afforded by the covering of the mother scale. On lifting a mature female during the reproductive period, a few larvae will always be found clustered close together in the small space around the pygidium. In this space, along with the larvae, the dry discarded amnion coverings are often found.

By treating some of these larvae with a 10 per cent KOH solution and then staining them with acid fuchsin, their mouth parts will show interesting degrees of internal transformation. In individuals recently born, the two pairs of bristlelike mandibles and maxillae are rolled up in two coils like the hairspring of a watch, with one on each side of the brain. This position of the mouth parts is also noticeable during the time that the larvae are still enclosed in their amnion coverings within the oviduct of the mother. The older larvae, which have just made their appearance and are still crawling about, have uncoiled their mouth parts in the form of long loops, each of which is contained within an internal sac-like structure, known as the crumena.

Soon after making their appearance, the young larvae crawl actively about and come to rest when a suitable feeding place has been selected. The preferred place for settling is beside the midrib or some other prominent vein of the leaf. The duration of this active crawling stage is variable, depending largely upon how soon a favorable feeding spot is located. The majority of the larvae have been noticed settling within the first 6 hours, but a few have been found crawling about, 24 hours after liberation.

Mortality was highest during this stage. In the writer's opinion the cause of this lies in the inability of the larvae to pierce the epidermis, resulting in death from starvation; the inability of the larvae to form a protective wax covering, thereby leaving themselves exposed to drying; and dropping off the leaf, resulting in inability to reach the host plant again.

After finding a suitable feeding place, the larva proceeds to pierce the epidermis with its mouth parts. The rostrum is closely pressed against the surface of the leaf so as to support and direct the rostralis, composed of the united mandibles and maxillae, into the tissues. The distal or free end of the rostralis gradually passes through the rostrum, causing the long loop in the crumena to grow smaller and smaller. The piercing is aided by a slowly rotating movement accompanied now and then by a rise and fall of the anterior portion of the body.



Very shortly after the insertion of the mouth parts, the whole dorsal surface of the larva becomes gradually covered with very fine, silvery white, wax threads. As a result of the rotating of the body, these wax threads coalesce and assume the form of a flattened cone or cicatrix which soon covers the whole body. The legs and the antennae are withdrawn beneath the body and the latter becomes more rounded in outline during the formation of the wax covering. Following this and up to the beginning of the first molt, the only noticeable changes are the gradual expansion of the insect beneath the wax covering and a compacting and darkening of the latter around the outside rim. During this whole period the insect remains unattached to its wax covering, which, when it is removed, is soon replaced by the secretion of a new one. Quayle<sup>(28)</sup> found that:

"In the case of some the covering was removed and the maximum number of new coverings formed was four. When the covering was removed three or four times, the insect usually died."

The first instar ends with the first molt. The duration of this stage was found to be about the same for both the red and the yellow scales, which had an average of 13 and 13.13 days respectively (see tables 2 and 3). A great variation in the degree of development of the individuals in the different lots was noted, evidently due to the fact that in some cases conditions were more favorable for rapid development. There was differentiation of males and females during this stage.

*First Molt*—Externally the first molt can be recognized by the outer rim or margin of the wax covering, which becomes clear-cut and more compact. If the insect is lifted during this period, it is found firmly attached to its covering, and the body itself is in a turgid condition. The ventral side of the insect, proximad to the leaf surface, is light brown in color and polished in appearance. A microscopic examination of the internal structures during this period, or just prior to the actual molt, shows the developing second stage lying within the body wall of the first stage. Again the coiled mandibles and maxillae are evident on each side of the mouth region. There is, therefore, a short period during the actual molting process and just before the insertion of the new second stage mouth parts, when no food is taken from the plant tissue.

The splitting of the larval skin usually starts in the region of the mouth and then follows along the sides. The dorsal skin is incorporated into the wax covering to which it adheres firmly; the ventral skin which carries the mouth parts, the antennae, and the legs, is pressed down on the surface of the leaf beneath the molted insect.

The molting period, as shown in tables 2 and 3, lasts from three to four days, being approximately of the same duration in both the red and yellow scales.

*Second Stage.*—After molting, the insect enters the second stage of its development.

That the first molt has been completed, is indicated externally by the extension of the wax rim, which is secreted around the margin of the former covering. On lifting this covering it is found that it may be removed readily from the insect, and remains more or less detached until the beginning of the second molt. It is during this stage that the scale coverings of the two sexes can be differentiated externally.

The female continues the rotating movement resulting in the addition of a circular rim to the covering of the first stage. This additional rim consists of a somewhat dull, compact, waxy secretion. Some interesting observations as to the secretion of this wax were made by placing a second-stage female, deprived of its scale covering, under a microscope and studying the flow of wax filaments from the abdominal pores. These filaments were at first excreted vertically, and upon attaining maximum length they fell over as a result of their own weight. They then became entangled in the pygidial fringe, because of the up and down movement of the pygidium. It appeared that the pygidial lobes and plates acted as the teeth of a comb, causing the tangled mass of wax filaments to be more closely united and formed into a ribbon which then passed out to form the waxy portion of the scale covering.

The early-stage male covering is found to be similar to that of the female except that the wax is lighter colored and does not seem to be of the same compactness and rigidity. Later, however, a decided elongation takes place primarily as the result of the cessation of the rotation of the body, and the continued secretion of the wax toward the posterior end of the body.

*Second Molt.*—The second molt is similar to the first. During this period, the female again becomes firmly attached to its dorsal covering, while the internal organs undergo transformation in preparation for the third or final stage. The coiled mouth parts are again evident, while the outer rim of the scale covering becomes clear-cut and well defined. Splitting or shedding of the old skin takes place along the sides of the body, the dorsal surface being incorporated in and firmly attached to the scale covering, while the ventral surface is pressed down on the leaf surface as in the case of the first molt. The duration of this molt was found to be about the same for both scales, the red showing an average of 3.63 days and the yellow 3.75 days.

During both the first and the second molt, there was a certain degree of mortality. This is evidently a result of the inability of the insect to break loose from its previous skin or the inability to uncoil its mouth parts so as to pierce the host tissue again.

*Adult Stage.*—Completion of the second molt brings the female to its final or adult stage. Before the production of young begins, two interesting subphases are to be noticed, which will be respectively called the prefertilization and the postfertilization stages.

Upon completion of the second molt, a further slow deposition of wax takes place until the external covering has attained its final dimensions. This stage can be easily recognized by the following characters:

1. Dorsal scale covering not firmly attached to the underlying female.
2. Slow rotating movement of body resumed in depositing wax rim.
3. New wax rim running around the margin of the older scale covering, soft and light gray in color.
4. On removing the dorsal scale covering, the exposed female is found to be pear-shaped, and in the case of *citrina* decidedly more yellowish in contrast to *aurantii*, which is more reddish.

It is during this stage that fertilization takes place, and the longevity of the insect depends upon how long after maturity fertilization occurs. Soon after fertilization has taken place the female enters its postfertilization stage.

During this stage the fertilized eggs develop rapidly. The outstanding changes that take place here can be briefly summarized as follows:

1. The rotation of the body ceases.
2. There is no further secretion of wax.
3. The body becomes firmly attached to the dorsal scale covering.
4. The body cuticula, especially on the dorsal side, becomes thickened, forming a fairly hard exoskeleton which is less elastic than in any of the previous stages. The cuticula is visible through the lighter wax area of the early third-stage wax rim. Together with the other previously incorporated molted skins, the dorsal scale covering takes on its characteristic color: brownish red in the case of *aurantii*, and yellowish green in the case of *citrina*.
5. When fully developed the thorax extends backward in a large rounded lobe on each side, projecting beyond the extremity of the abdomen, and giving the body a reniform shape.
6. The ventral portions of the molted skins of the previous stages are found closely pressed together on the surface of the leaf just below the adult female, and in comparison to those of the dorsal portions, are lighter, thinner, and more parchmentlike.

In many cases these ventral cast skins are found attached to the dorsal covering, with the result that it seems as if the female reposes within a baglike structure. This, however, is brought about after molting and is probably due to subsequent wax secretion, which glues the ventral skins to those of the dorsal covering. If this ventral scale is treated with KOH, the constituent skins fall apart, clearly indicating that the splitting of the skin during the previous molts had followed not only the general body margin, but often the margin of the lobes and plates as well.

The time that elapsed between the completion of the second molt and the appearance of the young was somewhat different for the red and yellow females; with the former this period averaged a total of 32.5 days and with the latter a total of 35.63 days, giving a difference of 3.13 days.

*Reproductive Period and Number of Young.*—Under a fairly high and constant temperature, 82° F, there was a continuous emergence of young over the whole reproductive period, which, under those conditions, lasted 60 days for both the red and the yellow species. The experiments were not extensive enough to furnish reliable data on the total possible number of young that might be produced, but they did show that both species produced young at about the same rate and in the same number.<sup>6</sup>

*Duration of Total Life Cycle.*—By comparing the respective life cycles, from free-moving larvae up to the time of reproduction, we find by referring to tables 2 and 3 that the cycle of the yellow runs from 61 to 68 days, and that of the red from 60 to 63 days, giving an average of about 65 and 61 days respectively, or a difference of about 4 days.

*Sex Ratios.*—Of the 521 red-scale adults procured during the second half of the year there were 319 females and 202 males. Of the 249 yellow adults procured during the same period there were 108 females and 141 males. One hundred red larvae and 70 yellow larvae were transferred during the early spring. Of the 65 red scales which reached maturity there were 19 females and 46 males. Of the 48 yellow scales which reached maturity, the females were even more decidedly in the minority, there being 2 females and 46 males. The preponderance of males in the last case is unaccounted for.

<sup>6</sup> The number of young produced by a single female varies greatly according to the food and temperature conditions, and to the technique employed by those engaged in conducting the experiments. One hundred and fifty young were produced by one female on a lemon fruit. There seem to be more young produced on the fruit than on the leaves.

## BREEDING EXPERIMENTS

Since parthenogenesis has been reported for various members of the Coccidae, a series of experiments was conducted to determine whether or not this might be the case with either *citrina* or *aurantii*.

Two lots of 50 red and 50 yellow free-moving larvae were placed upon previously banded leaves of two young potted orange trees. Each of these trees was then isolated in a sealed, insect-proof cage, so as to prevent any outside males from reaching the females. As soon as sex differentiation was evident all the males were removed, leaving a total of 30 red females and 35 yellow females in each cage. These females were kept thus isolated until most of them were dead, and in no case were any progeny produced, showing that parthenogenesis did not take place.

The active part which the male plays in fertilization was further brought out in a number of instances where the act of copulation was observed. The following notes were made in one case: Just after emerging from beneath its scale covering, the male moved actively around for some time until it eventually stopped in front of an early third-stage female, at which time copulation took place. The male withdrew a short distance, and began to wipe off its antennae with the aid of its fore legs. This process was kept up for about ten minutes while the movements became gradually slower and slower until all indications of life ceased. This observation, that is, from the time the male emerged until its death, lasted from 9:45 A. M. to 10:35 A. M., indicating the short life of the adult stage of the male.

In a number of cases the males took to flight immediately upon emergence, evidently seeking to fertilize females on adjacent leaves. Flight of the males was sluggish, taking place in gradually increasing circles. In no instance did a male fertilize more than one female, although the same female was fertilized by two or more males. The larger proportion of males appeared during the night, a condition to be expected as the male is of an extremely soft and frail nature, and could not survive exposure to direct sunlight and high temperatures.

A number of experiments were conducted with the object of determining whether or not interbreeding takes place between *aurantii* and *citrina*. As time would not permit more extensive investigations, the results presented here cover only one or two life cycles.

Because of their delicate nature and extremely short life, the males proved very difficult to handle and control in large numbers. Other methods eliminating direct handling had to be resorted to, and of these,

three types will be described. Insect-proof cages were used throughout so as to prevent any outside males from coming in contact with the females used in the experiments. A quarantine isolation room was used when it was necessary to remove any of the males or females.

*Experiment A.*—Six leaves of a young potted orange tree were cleaned and their petioles banded with paraffin wax. Leaves 1, 3, and 5 were each colonized with 50 active red-scale larvae, and leaves 2, 4, and 6 with yellow-scale larvae. As soon as the sexes could be distinguished, the red males and the yellow females and the paraffin bands on the petioles were removed. In other experiments of the same type the red females and the yellow males were removed. To serve as checks, only the males of one type of scale were removed, leaving both the males and females of the other type. The results of these experiments are briefly indicated in table 4.

TABLE 4

## RESULTS OF INTERBREEDING EXPERIMENTS

Experiment	Matings	Results
A 1.....	R♀ × Y♂	No progeny produced
A 2.....	Y♀ × R♂	No progeny produced
A 3 (Control).....	R♀ × Y♂	Progeny from Y × Y only, none from
	Y♀ × Y♂	R × Y, indicating crossing only between
		yellow females and yellow males
A 4 (Control).....	R♀ × R♂	Progeny from R × R only, none from
	Y♀ × R♂	Y × R, indicating crossing only between
		red females and red males

*Experiment B.*—Four small trees were alternately colonized as follows: trees 1 and 3 with red larvae, and trees 2 and 4 with yellow larvae. The males in all four cases were removed as soon as this was possible. Oranges infested with large colonies of healthy yellow scales were placed in the cages containing trees 1 and 3, in such a manner that while the males could escape and reach the red females, the young active yellow larvae from the fruit were unable to reach the trees. With trees 2 and 4 the same was repeated, using fruit infested with red scale. In no case were young produced, indicating that no interbreeding had taken place.

*Experiment C.*—Colonization of four small trees with red and yellow larvae was duplicated as in Experiment B. Later when sex differentiation was possible, the following removals were made:

- (1) All red females from tree 1, leaving red males.
- (2) All yellow males from tree 2, leaving yellow females.
- (3) All red males from tree 3, leaving red females.
- (4) All yellow females from tree 4, leaving yellow males.

Trees 1 and 2 were then caged together, and similarly trees 3 and 4, thus allowing ample opportunity for any interbreeding to take place if such were possible. However, as in the two former experiments, no interbreeding took place.

### METAMORPHOSIS OF THE MALE

*First Stage.*—The metamorphosis of the Diaspine scales is more anomalous than that of any other insect. During the first nymphal stage the two sexes are indistinguishable. Both possess legs, antennae, and eyes, all of which are lost during the first molt. The female then continues with its gradual metamorphosis while the male follows an altogether different development, very similar to a complete metamorphosis. After its second molt, the male loses its functional mouth parts and gradually acquires a new set of legs, antennae, and wings. The legs and antennae are derived from histoblasts, or imaginal disks, which are similar to those found in insects with a complete metamorphosis, while the wings are developed externally, indicating a gradual development. The type of metamorphosis which the male undergoes is, therefore, neither strictly complete nor gradual.

The external characters of the four different instars of the male of *Aomidiella aurantii* have been fully described by Quayle<sup>(28)</sup> and Berlese and Leonardi,<sup>(1)</sup> and as the descriptions of these authors are identical to those of the writer for *citrina*, the following discussion will be limited to the development of the male appendages. In no instance was the presence of histoblasts noticed in the first-stage larvae, probably as a result of the expected minuteness of their formative cells during this stage. Matters were further complicated as there was no way of determining whether a male or female larva was under examination. The second stage of the male will, therefore, be taken as the starting point of the internal development of the appendages.

*Second Stage.*—About two days after the first molt the histoblasts of the antennae and the legs are noticeable (fig. 1A). The antennal histoblasts are situated at the extreme anterior portion of the cephalic end of the body, while those of the legs are found in the ventral thoracic region. These histoblasts first appear as thickenings of the hypodermis and later take on the form of round pockets or buds. Each bud forms the

center of a so-called peripodal cavity, which, as development proceeds, eventually opens, while the bud itself evaginates, sending out a finger-like process. These processes which represent the rudimentary appendages, point anteriorly in the case of the antennal and fore-leg histoblasts, and posteriorly in the case of the middle and hind-leg histoblasts. The wings gradually arise from buds on the lateral margins between the dorsal and ventral surfaces of the body.

The first pair of accessory eyes is situated posterolateral of the antennal buds and the second pair is found on the ventral cephalic surface, opposite each end of the brain. During this stage the eyes are present as dark-violet diffused areas. Just before molting, the appendages have attained approximately one-third of their final length. At about the same time the thoracic muscles begin to show plainly on the dorsal surface.

Transformation into the following or prepupal stage takes place rapidly so that just prior to molting it is found lying within the body wall of the second stage and separated from it by a margin filled with liquid. Actual molting takes place by a contraction, followed by a sudden expansion of the new internal prepupal body, causing the cuticula of the second stage to split at the cephalic end and to be pushed backward toward the abdomen where it can often be seen projecting from underneath the scale covering. During this molt the mouth parts are lost and not replaced during the subsequent stages.

*Prepupal Stage.*—The prepupal stage (fig. 1B) is marked by the further development of all the appendages. The latter are clearly seen enclosed within a thin sheath, with the joints, indicated by cross divisions, becoming more and more apparent. Schmidt,<sup>(30)</sup> working on the male of *Aspidiotus nerii*, was of the opinion that the primary object of this stage is to supply the necessary material at the right place, and to make, through molting, sufficient room for the subsequent stages. The body gradually takes on the shape of the final stage, and in addition, the histoblast which, upon evagination, will give rise to the genital organ or style, is clearly seen toward the tip of the last abdominal segment. At the end of this stage, the appendages attain approximately two-thirds of their final dimensions. The molting process is similar to that of the second stage.

*Pupal Stage.*—During the earlier portion of the pupal stage (fig. 1C), the joints of the different appendages become clear-cut; the lighter divisions marking the segments is not clearly seen until shortly before the final molt. The bristles on the legs and antennae are difficult to detect at first, but become clearer and better defined with the formation of the final cuticula. The evaginated style and all the other appendages are



clearly seen enclosed within their thin, chitinous sheaths; the wings are collapsed and folded within their sacs. The eyes have changed from diffused areas to definite areas which they occupy in the adult male. The last or final molt produces the fully developed male, which remains under the shelter of its scale covering for a day or so longer before emerging.

## EXTERNAL MORPHOLOGY

Because of its economic importance and wide distribution throughout the citrus-growing sections of the world, *Aonidiella aurantii* has attracted the attention of many workers, and the external characters of the female have been described in detail more than once. With reference to *citrina*, no separate description has been made, as workers generally have taken the latter to be structurally identical to *aurantii*.

However, to acquaint the reader with the female structures upon which the identification of this species is based, and in order to facilitate a better understanding of those characters which, in the course of this study, were found to be specific and constant for *citrina* but not for *aurantii*, a résumé will be given, embodying the original description of Maskell<sup>(22)</sup> and also the subsequent ones of Comstock<sup>(5)</sup> and Brain.<sup>(2)</sup> No constant morphological differences could be detected in the immature stages of either of the two scales, and as these were fully described by Quayle,<sup>(28)</sup> further descriptions will be omitted in this discussion. Additional notes on the adult male of *citrina*, and its development, will be given under a separate heading.

For the study of the external chitinous structures, the standard methods described by Gage,<sup>(10)</sup> Ferris,<sup>(9)</sup> and Brain<sup>(2)</sup> were employed; of these the 10 per cent KOH treatment and subsequent staining with either acid or basic fuchsin proved the most satisfactory. Rapid examinations of unstained and live specimens were made by mounting in glycerin. All measurements were made with the aid of a standard eyepiece micrometer.

*Adult Female of Aonidiella Aurantii.*—The scale covering is nearly circular, slightly broader (1.9 mm) than long (1.77 mm), with thin, flat margins and the central area flatly convex, and generally appears shiny or polished. The reddish color is due to that of the female insect beneath the scale. The exuviae are regularly central, and are covered by a thin layer of secretion. A small prominent spot with a concentric ring of whitish waxlike substance is found in the center of the larval exuvia. The ventral exuvia consists of the lower half of the molted skins of the previous stages; however, it varies greatly in texture, being of a grayish

white, parchmentlike character, often fused with the inner surface of the dorsal scale covering.

The body is orange red. It is reniform in shape as a result of the lateral margins of the body, which extend backward as two large rounded lobes, one on each side of the retracted abdomen. The body is flat beneath and slightly convex above. The abdomen is terminated by a flattened chitinous region or pygidium, composed of several fused segments. Antennae are absent, except for two minute papillae or antennal tubercles, set well back from the mouth parts and each carrying a fine, slightly curved spine. The derm is membranous throughout, except for the pygidium.

The pygidium (figs. 2*C* and 2*D*) is broadly rounded. The anus is on the dorsal side of the pygidium, and the genital aperture on the ventral side. On each side of the dorsal median line, about 17 tubular ducts are found; their openings are arranged more or less in three lines. Circumgenital glands are absent. Green <sup>(11, 12)</sup> has directed attention to the possible connection between the circumgenital glands and oviposition. Thus in those species which are viviparous, the glands as a rule are wanting, while they are present in all oviparous species, as stated by Imms <sup>(14)</sup>

On the pygidial fringe there are three well developed lobes: L1, L2, and L3. L1 and L2 usually are distinctly notched on both margins, the notch on the outer margin of L2 being more pronounced. The notch on the mesal margin of L1 is often nearer the distal end of the lobe than that of the outer margin.

The plates are found between the lobes, which they exceed in length. They are arranged as follows:

There are two plates between the median pair of lobes (L1-L2). These are deeply fringed on their distal margins only.

There are two plates between lobes L1 and L2, two between L2 and L3, and three between L3 and the margin of the body. These are all fringed on their outer margins.

The first plate laterad of the second lobe, and the three plates laterad of the third lobe, are each deeply bifurcated, each bifurcation being fringed on the outer margin.

*Adult Female of Aonidiella Citrina.*—The scale covering is similar in structure and outline to that of the red-scale female, except that it is slightly flatter and smaller, having an approximate width and length of 1.725 mm and 1.58 mm respectively. (For further comparative dimensions, see table 5.) The most outstanding difference, however, is the yel-

TABLE 5

COMPARATIVE MEASUREMENTS OF ADULT FEMALE SCALE COVERINGS; MILLIMETERS

<i>Aonidiella aurantii</i>				<i>Aonidiella citrina</i>			
Entire scale covering		Longest diameter of second-stage incorporated skin	Longest diameter of first-stage incorporated skin	Entire scale covering		Longest diameter of second-stage incorporated skin	Longest diameter of first-stage incorporated skin
Width	Length			Width	Length		
2.025	1.800	0.810	0.405	1.800	1.575	0.720	0.315
1.800	1.710	.720	.405	1.575	1.485	.675	.315
1.935	1.820	.810	.360	1.710	1.575	.675	.315
1.890	1.820	.810	.360	1.800	1.575	.675	.360
1.800	1.800	.810	.360	1.575	1.575	.675	.360
1.935	1.800	.810	.405	1.820	1.530	.720	.360
1.935	1.890	.855	.360	1.935	1.710	.765	.360
2.025	1.710	.810	.405	1.665	1.575	.720	.315
1.890	1.890	.765	.405	1.800	1.710	.720	.360
2.025	2.025	.810	.405	1.800	1.800	.765	.405
1.890	1.800	.810	.360	1.710	1.350	.630	.405
1.710	1.485	.810	.405	1.820	1.665	.810	.315
1.890	1.665	.810	.405	1.575	1.350	.630	.360
2.025	1.890	.810	.405	1.665	1.575	.765	.405
1.890	1.575	.810	.315	1.710	1.575	.765	.360
Av. 1.900	1.777	0.800	0.384	Av. 1.725	1.581	0.714	0.354

TABLE 6

COMPARATIVE MEASUREMENTS OF PYGIDIAL LOBES;  
MILLIMETERS

<i>Aonidiella aurantii</i>			<i>Aonidiella citrina</i>		
L1	L2	L3	L1	L2	L3
0.011	0.011	0.015	0.015	0.015	0.015
.015	.015	.015	.019	.015	.015
.015	.015	.011	.023	.019	.019
.011	.015	.015	.023	.019	.019
.015	.011	.015	.019	.019	.019
.015	.015	.015	.019	.019	.019
.015	.015	.011	.023	.023	.023
.015	.011	.015	.019	.015	.015
.015	.015	.015	.023	.019	.019
.015	.015	.015	.019	.019	.019
.015	.015	.011	.021	.019	.019
.015	.011	.015	.019	.019	.019
.015	.015	.015	.019	.019	.019
.015	.015	.011	.019	.019	.019
Av. 0.014	0.014	0.014	0.020	0.018	0.018

low color of the covering, which differentiates it easily from that of the red scale.

The body is identical in shape to that of the red female, but different in its clear, yellowish-green color.

On the pygidial fringe (fig. 2*A*), lobes L1, L2, and L3 are well developed. Laterad of the last three plates, the margin of the fourth abdominal segment forms a well-defined triangular process or lobe. The surface of the latter varies from serrate to smooth, as does that of the other lobes. This lobe was found to be constant for a large number of individuals examined, thus permitting mounted specimens to be differentiated.

Considering the three main lobes, the writer's attention was drawn to their evident slender appearance. Measurements were taken which showed them to be appreciably longer than the corresponding ones of the red females. Table 6 presents measurements taken at random of 14 red and 14 yellow females. Furthermore, as shown in figure 2, lobes L2 and L3 of the yellow females are not so deeply notched as the corresponding ones of the red females.

The structure, location, and bifurcation of the plates are similar to those of the red scale. The pair of plates situated between the median pair of lobes (fig. 2*B*) appear more fringed than those of the red scale. However, as a result of a great variation within the individuals studied, no great importance was attached to this difference.

*Adult Male of Aonidiella Aurantii and Aonidiella Citrina.*—In addition to the studies conducted on the females of *Aonidiella aurantii* and *A. citrina*, special attention was also given to the external morphology of the males of both of these species. The object was to determine whether or not there were sufficient constant differences by which the two species could be segregated. A careful examination failed to show any differences upon which a satisfactory and absolute differentiation could be based. However, as a result of these studies, additional data on the external morphology of the male of *Aonidiella citrina* (fig. 3) were obtained which will be briefly presented in the following pages.

The general description given by Quayle<sup>(28)</sup> for the male of *Aonidiella aurantii* will cover equally well that of *A. citrina*. Quayle states:

The adult male has a wing expanse of 1.5 mm.; length exclusive of style .6 mm.; style .22 mm.; color orange yellow; antennae ten-jointed, the first two segments being much shorter and thicker than the others. The comparative lengths beginning with the proximal one, are as follows: 5-4-17-20-20-20-18-15-13-17. Total length 0.5 mm. The antennae are light colored with some yellow pigment. On all the joints excepting the first two are rather long hairs. The lateral pair of eyes are dark brown

and situated just laterad of the antennae. The ventral pair of eyes are much larger and closer together and situated more posteriorly. The legs, excepting coxae which are yellow, are glassy white, and the tarsi light brown. The thoracic band is of a light brown color. The halteres are club shaped, with the slender hook arising from the tip of the club.

As the above description is rather brief, the following additional descriptions of the cephalic, thoracic, and abdominal regions are given. By reference to figures 3, 4, 5, and 6, the more important structural characters of these can be easily followed. Some of these structures are named according to homologous ones worked out by Berlese and Leonard<sup>(1)</sup> for *Aspidiotus hederae*.

Figure 4 represents a semidiagrammatic aspect of the ventral side of the head; the dorsal structures are indicated by broken lines. The head is more or less triangular in outline with the anterior end somewhat compressed dorso-ventrally. It is broadest across the posterior or basal end, which is marked by a fairly well defined suture.

On each side of the head and well within its margin, the malar ridges start at a point intermediate between the dorsal accessory and the ventral accessory eyes and the primary eye, and extend in a posterior direction. These malar ridges are strongly chitinized, slightly curved, broadest through middle, and tapering toward each end. In the immediate vicinity of the basal suture, each turns directly inward and upward and fuses with its corresponding mate from the opposite side on the median line of the head, thus giving rise to a ridge which is known as the ocular apophysis. The latter has a slightly broadened apex and terminates in the region between the two ventral accessory eyes.

On the dorsal surface of the head, situated between the dorsal accessory eyes, the peculiarly shaped cephalic ridge is found. Situated at the base of the ocular apophysis, a fleshy tubercle, representing the rudimentary mouth parts, is found. In cross sections the esophagus terminates in this tubercle, showing no actual opening to the outside. Viewed ventrally, each of the dorsal accessory eyes is concealed by a ridge or scape, which extends posteriorly from the base of the first antennal segment. The bases of the antennae are separated by a row of well defined and unpaired spines which are situated in a shallow furrow.

As pointed out before, there are two pairs of accessory eyes and one pair of primary eyes. The ventral accessory eyes appear as two large, ovoid, closely approximated areas situated on each side of the median line in the caudal half of the ventral aspect of the head. The circular and dark-brown dorsal accessory eyes lie just caudad of the base of the antennae on the dorso-lateral line of the head. The primary eyes can be

seen best from the ventral side. They are two colorless, beadlike projections with translucent tips, situated on the lateral surface of the head, opposite the cephalic half of the ventral eyes, and immediately posterior to the dorsal accessory eyes. It is remarkable that the primary eyes can best be seen from the ventral side, even though they lie just behind the dorsal eyes.

The dorsal and ventral accessory eyes are similar in structure (fig. 1*D*). Each eye is surrounded by a groove which is formed as the result of the pushing upward of the body wall into a ridge. The lens of the eye is a large, spherical, globular, transparent body, consisting of a homogeneous substance covered by a thin layer of chitin. Between the lens and the visual rods is the corneal hypodermis, which occurs as a thin flattened layer. The iris surrounds the corneal hypodermis and consists of a layer of highly pigmented cells. Next to the corneal hypodermis is a crescent-shaped area filled with visual rods. The latter are translucent and slender, tapering slightly toward their distal ends, and separated from one another by a dark seam. The retina which follows is made up of retinal cells regularly arranged opposite the bases of the visual rods. The distal ends of the retinal cells are contracted and prolonged into nerve fibers which unite to form the optic nerve.

The primary eyes are very simple in structure and differ from the accessory eyes in that they do not possess the corneal hypodermis, iris cells, and visual rods. The lens consists of a slight thickening of the cuticula with an outer convex surface.

The *prothorax* (fig. 4) is mostly soft and fleshy, as no sclerites are developed. In each of its lateral ventral halves there is a large and well developed trochanter which articulates posteriorly with the first pair of coxae.

Of the three thoracic regions, the *mesothorax* (figs. 5 and 6) is the largest, showing a well developed tergum, pleuron, and sternum. Figure 5 represents a dorsal aspect of the mesothorax.

The scutum occupies practically half of the entire mesothoracic area. Anteriorly it is bounded by a chitinous ridge which on its inner side, forms a loop enclosing a humplike area, called by Berlese and Leonard<sup>1</sup> "gobba del mesotorace"—the prominence of the mesothorax. Posteriorly the scutum is separated from the scutellum by the broad, dark-brown thoracic or interseutellar band.

The posterior half of the mesothorax is occupied by the heart-shaped scutellum. The pleuron consists of circular sclerites with the wings and halteres arising between the tergo-pleural sutures.

Figure 6 represents a ventral aspect of the mesothorax. The mesosternum appears like a chitinous rectangular frame with an internal fork-like structure, the function of which is evidently to serve as a means of attachment for the thoracic muscles. A chitinous ridge or linear sternite is found on the median line of the prosternum.

The *metathorax* (fig. 6), like the prothorax, is fleshy and poorly defined, especially in specimens treated with KOH. On the ventral side of the metathorax two long chitinous processes, called the epimera by Berlese and Leonardi,<sup>(1)</sup> run posteriorly between the bases of the halteres and the third pair of coxae. The second pair of coxae are attached to similar but shorter structures from the pleural regions. The position of the two pairs of spiracles in the mesothorax and the metathorax is also indicated in figure 5.

The *abdomen* (fig. 3) consists of eight segments and is terminated by the prominent style or genital sheath, which encloses the aedeagus.

*Mouth Parts of Female.*—The gross arrangement of the mouth parts (figs. 7B and 7C) of *Aonidiella aurantii* and *A. citrina* is homologous to the corresponding arrangement of the generalized homopteran, and does not show any marked differences from the Coccidae worked out by Mark,<sup>(21)</sup> Putnam,<sup>(27)</sup> Moulton,<sup>(23)</sup> Berlese and Leonardi,<sup>(1)</sup> and Childs.<sup>(3)</sup> The mouth parts are essentially adapted for piercing and sucking, the food being entirely liquid.

In this discussion, the descriptions will be limited almost entirely to the more important parts which have a direct bearing upon the structure and function of the mouth parts.

The chitinous, boxlike framework (fig. 7B) is clearly visible on the ventral median surface of the female, especially in those individuals which have been treated with KOH and subsequently stained. Its function in the main is twofold: First, it is very important in serving as a means of attachment for the powerful muscles which govern the pharyngeal sucking and salivary apparatus. Second, it protects and supports the underlying organs.

The framework is broadly triangular in shape, as a result of four pairs of chitinous arches which enter into its construction. Its base is formed by a smaller dorsal and larger ventral portion, called respectively by Mark,<sup>(21)</sup> the "arcus-superior" and the "arcus-inferior." The portions which form the laterals of the triangle have been called by the same author, the "costae"; the dorsal ones, costae superiores, and the ventral ones, costae inferiores. These structures are fused together at their extremities, forming the rigid boxlike structure. At the apex which points

posteriorly, sufficient space is left for the passage of the buccal setae, pharynx, etc.

There are four extremely long, slender, bristlelike setae (fig. 7B), representing the two pairs of modified *mandibles* and *maxillae*. Together they form the rostralis, in the formation of which the maxillae play the more important role. The two maxillae are closely united, leaving a small lumen which serves as a passage through which the liquid food from the host to the pharynx may pass; on their exterior they are protected by the mandibles, situated on each side. At its proximal part, each seta gives rise to a conical body, called the "conus" by Mark.<sup>(21)</sup> Distally each pair passes through the anterior opening of a curious, highly chitinated, baglike apparatus, called the clavus. The clavus gradually tapers, forming, with its corresponding arm from the opposite side, a common apex. Within this apical portion, the two pairs of buccal setae come in close contact with one another, giving rise to the rostralis. The rostralis runs posteriorly, making a loop within the crumena. During the larval stage, the crumena is extremely well developed, extending as far back as the second abdominal segment. In the case of *Warajiococcus* Kitao<sup>(15)</sup> describes the crumena as follows:

A tubular passage, which extends back just below the thoracic ganglion and terminates in a blind sac, embedded in the connective tissue without lying free in the body cavity, as stated by some writers. In cross section it is dumb bell shaped, its wall consisting of three layers, much as in the integument. The inner layer, exclusive of its innermost part, is fairly thick and weakly chitinated. Next comes the middle hypodermal layer which is very thin. The outer layer is represented by an inconspicuous basement membrane.

From the crumena, the rostralis passes upward and so through the rostrum. Just before birth, and during the subsequent molts, the mandibles and maxillae are found to be coiled up, a pair on each side of the brain. The mandibles and maxillae evidently arise from their respective imaginal disks; the latter lying beneath that of the former. These disks are of hypodermal origin, forming, during each molt, a new set of mouth parts.

The *rostrum* (fig. 7C) is but a modified, one-segmented labium, conical in shape and attached at its base to the ventral side of the thorax. The lower side of the rostrum forms, as a result of folding, a closed canal in which the setae lie. Within the rostrum and attached to chitinous plates are a few muscles, undoubtedly used when the setae are forced into the host tissue.

The *salivary pump* (fig. 7C) lies beneath the anterior part of the pharynx and close to the point of junction of the two arms of the clavus. It



consists of a highly chitinized, cuplike structure or cylinder, carrying an inverted top in the form of a "plunger." This plunger is flexible, with a heavy, chitinized, Y-shaped tendon, to the base of which are attached some powerful retractor muscles. There is no doubt that these muscles cause the rapid up and down movement of the plunger. A small flaplike membranous ridge runs around the middle of the plunger. The function of this flap or ridge is valvular, cutting off, on the down stroke of the plunger, the flow of saliva from the glandular duct.

The paired salivary branches unite to form a common duct, which enters the cylinder through the anterior part of the dorsal surface. Posteriorly the lateral sides of the cylinder curve inward, leaving a small lumen, which communicates with the general mouth cavity. The latter is bounded by the lingua (arcus superior) and the labrum (arcus inferior), close to the extremities of which the bases of the mandibles and maxillae are found.

There seems to be some doubt as to the actual function of the salivary pump. In the case of certain heteropterous forms, studied by Muir and Kershaw,<sup>(24)</sup> its mode of operation is described as follows:

"The plunger being retracted by the muscles, draws the saliva from the ducts into the syringe-barrel. On relaxation of the muscles, the natural elasticity of the plunger performs the return stroke, closing the valve and forcing the saliva past the tongue on the base of the labium."

Berlese and Leonardi,<sup>(1)</sup> who studied several species of coccids, is of the opinion that the pump draws the saliva from the large paired glands, instead of only from their common duct. Childs,<sup>(9)</sup> on the other hand, states that:

The rule for most insects with comparable organs (large paired glands, ducts, and openings into the cylinder) is that these glands, as a result of an internal pressure caused by a continual secretion of the cells from within, or from muscular action, force the juices out. In the case of *Epidiaspis puricola*, no muscles are to be found that could perform this function, and, from the make-up of the glands themselves, they seem to the writer to be admirably adapted to operate through a pressure formed from within. Again, from the make up of the long, slender, four-pieced proboscis, it would seem to be impossible to pump saliva into the plant tissues, for pressure from the inside would disrupt the tube. Necessarily, therefore, if there is a passage of fluid down this setal arrangement it would have to be done with little or no pressure.

As a result of a number of experiments conducted upon live specimens, the observations of the writer seem to agree more with those of Muir and Kershaw than with those of Childs. By careful manipulation, so as not to damage or break off their rostrali, early third-stage females were removed from the host tissue and placed upon slides directly under

the microscope. Immediately, a clear, colorless drop of saliva was formed at the distal end of the rostralis, indicating that saliva does pass down the latter organ and so into the tissues of the host. That the food juices pass up and down separate canals within the rostralis was further demonstrated by placing the tip of the latter in a drop of water soluble stain. The stain was found to pass up the rostralis, while the continued flow of saliva was indicated by a clear zone within the drop of stain.

By taking into consideration the length and chitinous nature of the rostralis, its position within the host tissues, and the rapidity with which the saliva passes down its whole length, one must come to the conclusion that a certain amount of pressure is derived from the highly developed salivary pump. Passage of the saliva from the glandular cells into the ducts is probably caused, as has been suggested, by an internal pressure, the saliva being drawn further into the cylinder by the upward stroke of the plunger. Disruption of the setal tube within the plant would hardly result from internal pressure, as any such tendencies would be sufficiently equalized by the surrounding tissues. The toxic action of the saliva of this species upon the chlorophyll of the host leaf is clearly indicated by the appearance of a yellow streak, running from underneath the side of the settled insect.

The *pharynx* (fig. 7C), which opens into the mouth cavity, is somewhat swollen and provided with thick, irregular, chitinous walls, which abruptly thin down as they come to form the walls of the true esophagus. To these thick walls, called "uva" by Mark,<sup>(21)</sup> are attached a series of powerful divaricator muscles passing through the labrum, with their other extremities attached to the arcus inferior. The function of these muscles is to contract and expand the pharynx, thereby causing the liquid food to be sucked up through the rostralis and mouth cavity and so into the esophagus.

#### INTERNAL ANATOMY OF AONIDIELLA CITRINA

Morphologists have neglected the Diaspine group in the past, directing their studies more particularly to the generalized or unarmored coccids. Berlese and Leonardi<sup>(1)</sup> and Childs<sup>(3)</sup> are the only workers who have contributed outstanding papers on the internal anatomy of the Diaspidinae. The former worked on *Lepidosaphes vulva*, and the latter on *Epidiaspis piricola*. It is hoped, therefore, that the following observations, although not agreeing in all respects with those of the above-mentioned authors, will contribute something to a better understanding of the internal structures of this very interesting group.

For the investigation of the internal anatomy the usual methods of serial sections were employed. Sections varying from  $3\mu$  to  $10\mu$  were cut and subsequently stained on the slide. For staining, both Delafield's haematoxylin and Mayer's haemalum were used, with eosin as a counter-stain. For sectioning material, early third-stage females were preferred to the adult or full-grown females, because the latter were found to tear considerably as a result of their hard, inseparable scales, chitinous cuticula, and the resistance offered by the fully developed ovaries. The large ovaries with their developing embryos also tended to obscure the other finer anatomical structures. The majority of these difficulties became negligible with early third-stage females, as their scale coverings could be removed with ease, thus leaving only a soft cuticula with which to contend. The ovaries were still in a developmental stage, and were thus not sufficiently tough to offer any difficulties in cutting. Direct dissections were also attempted, but again the fan-shaped reproductive organs occupying the greater part of the body cavity proved the greatest hindrance in making such dissections a success. The majority of the drawings were made with the aid of a camera lucida.

*Integument.*—The structure of the integument is similar to that of most insects. The whole body is covered by a cuticula which, in well stained sections, shows two layers, namely, the epidermis and the dermis, the former being much thinner than the latter. Next beneath the cuticula is the hypodermis which rests upon an extremely thin and hardly noticeable basement membrane. The hypodermis forms a continuous layer of cells which change from a columnar type in the median part of the body, to a fusiform type at both the anterior and posterior extremities.

*Digestive System.*—Of all sucking insects, perhaps the Homoptera offer the greatest variation in the form of their digestive systems, most of them showing an organization which greatly attracts the interest of the research worker. Even within the Coccidae we find the digestive system diverging from a more or less straight duct, as in the Disapidinae, to those which assume a convoluted or recurrent course as has been demonstrated for members in the other subfamilies such as the Lecaniinae, Xylococcinae, Coccinae, etc.

Passing anteriorly from the mouth cavity, the *pharynx* runs into the thinner walled esophagus (figs. 7C' and 8B), which extends further forward until it reaches the base of the cephalic ganglion. It then curves sharply backward, running between the circumesophageal commissures into the thorax where it joins the ventriculus. At the point of junction, the cardiac valve is found. The esophagus is narrow and of more or less the same width throughout. Its wall consists of a fairly thick, chitinous

intima and an epithelium carrying the esophageal glands in the region where the esophagus curves toward the ventriculus.

Berlese and Leonardi<sup>(1)</sup> state that the ventriculus or *stomach* is a closed sac in *Lepidosaphes vulva*, entirely disconnected from the hind intestine except for two extremely thin and ductless ligaments which stretch from the posterior end of the stomach to the anterior end of the rectum. Digestion, according to them, takes place as a result of the action of the digestive juices rendering the food contents capable of passing by osmosis into the main haemocoelic cavity; the waste substances are removed from the latter by means of the greatly enlarged Malpighian tubes.

Childs,<sup>(3)</sup> on the other hand, in discussing the relation of the stomach to the hind intestine in *Epidiaspis piricola*, gives the following inadequate description:

"However (in referring to the findings of Berlese), the writer finds some sections in his series that show what can hardly be denied to be direct connections."

As no further information was available from the rather indistinct illustrations, the writer is still at a loss to explain what Childs referred to as "direct connections"; that is, whether they occur between the hind intestine proper or the Malpighian bulb, a structure which will be described later.

From the respective findings of these two authors, it is evident, therefore, that they are not in agreement on a morphological point, which is of sufficient importance to divide the Diaspine digestive system into two separate types, depending on whether or not a connection exists between stomach and hind intestine. It is possible that certain minor variations do occur in the different species studied by these authors, but considering the digestive system as a whole, one is inclined to believe that the Diaspidinae possess a constant type peculiar to this group. In the discussion which follows it will be shown that a direct connection does exist between the fore and hind intestine.

In *citrina*, as a result of a constriction in the wall of the anterior part of the ventriculus (fig. 8B), there is found around the cardiac valve, a small antechamber, the function of which is probably comparable either to that of a sucking stomach or a filter chamber. Posteriorly the stomach widens, taking on the characteristic baglike appearance. The digestive epithelium of the stomach consists of a row of irregular cells, with an occasional one greatly enlarged. These large cells, carrying correspondingly large nuclei, are probably the active, secreting ones, while the smaller ones are still in a developmental stage. These cells are all poorly

demarcated and void of any protrusions into the general lumen of the stomach, giving the surface of the enteric epithelium a smooth and wavy appearance. This type of structure immediately suggests, in part at least, Berlese's osmotic theory of digestion and assimilation. The outer ends of the epithelial cells rest upon an extremely thin basement membrane followed by the muscular layers.

At its ventral posterior end (fig. 8C), the ventriculus is found to be definitely connected with a highly nucleated, ovoid body called the Malpighian bulb (bulbo del peduncolo dei Malpighiana) by Berlese and Leonardi<sup>(1)</sup> Viewed from a basal aspect this bulblike body, or swelling of the small intestine, is easily recognized not only by its nucleated appearance, but also by its median position above the junction of the two Malpighian tubes. From this aspect the body also seems to be actually separated from the stomach, a condition which probably led Berlese to believe that it had no connection with the stomach. The actual connection, however, is not readily detected because it occupies only a small area between the anterior-ventral surface of the Malpighian body and the stomach. The junction of the two well developed Malpighian tubes at this point also tends to obscure a clear view of the connection proper. In sections the lumen of the connection appears to be filled with a porous protoplasmic tissue, as a result of the proximity of the epithelium of the stomach and the Malpighian bulb.

That digestive activity does take place within the Malpighian bulb, is indicated by its well developed epithelial layer which consists, as in the stomach, of irregular, nucleated cells, the outlines of which are not clearly defined. A short, narrow and slightly curved duct leads from the posterior end of the Malpighian body, connecting the latter with the anterior extremity of the rectum. This duct is readily recognized by its profusely nucleated cells which are closely united around a well marked lumen.

The *rectum* (fig. 8A), projecting posteriorly, is a comparatively wide and straight passage lying in the median portion of the body. It gradually tapers down, assuming the character of a narrow canal which shortly opens to the exterior through the anus situated on the dorsal side of the pygidium. On one side there is a caecum which was at first mistaken for a rectal fold. Kitao<sup>(15)</sup> mentions a similar structure in the case of *Warajicoccus*. Structurally the rectum is very simple, presenting only a thin epithelium, the cellular structure of which is extremely hard to differentiate.

There is only a single pair of large, cylindrical *Malpighian tubes* (figs. 7A and 8C) lying in the main body cavity. From their point of fusion

between the stomach and the hind intestine, the tubes run laterally for a short distance, turn abruptly upwards and then run posteriorly, almost to the caudal end of the body. Their posterior ends are attached to the sides of the rectum, a short distance forward of the anal aperture, by thin filaments. Structurally the tubes consist of large, polygonal cells, and have reticulated cytoplasm and distinct nuclei. These cells stand in conjunction with a central threadlike lumen which runs anteriorly, ultimately to open into the small intestine between the stomach and the Malpighian bulb.

*Reproductive System.*—As a result of the outward resemblance of the reproductive system of the female coccid to that of other insects, past investigators have described it as comparable to the usual insect type. According to Emeis,<sup>(7)</sup> however, such a comparison, except perhaps in the widest sense, is hardly warranted. Until recently the origin of the three ovarian elements, namely the nurse cells, egg cell, and epithelial cells, was very obscure. Leydig,<sup>(17)</sup> one of the earliest investigators, working on *Coccus hesperidum*, claimed that the nurse cells, egg cell, and epithelial cells must have arisen from undifferentiated germ glands. Leuckart<sup>(16)</sup> and Lubbock<sup>(19)</sup> came to the conclusion that these three elements are all originally undifferentiated cells of the germ rudiment. Emeis<sup>(7)</sup> failed to show whether the epithelial cells, from which the egg and nurse cells develop, came from the primordial cells or from the original mesoderm.

Shinji,<sup>(31)</sup> however, in his work on the morphology of the coccids, showed conclusively that the three ovarian elements are derived from the primordial germ cells. According to Shinji, the germ cells in the ovary give rise to a mass of oögonia by means of mitosis. After undergoing a resting period, a few oögonia, situated along the periphery of the ovary, undergo a last division, giving rise to four oöcytes. Shortly after this a protoplasmic substance is secreted toward the proximal end. These three secretory oöcytes nourish the single oöcyte located below, and thus become the so-called nurse cells. The subsequent history of the oögonium or ovariole is described as follows, by Shinji:

The cytoplasm areas of the fast growing nurse cells soon come into contact with one another. Being colloidal in nature, the nutritive substances secreted by the nurse cells elongate in the direction of the least resistance, which is in this case toward the egg nucleus. . . . The nutritive substance, which is elaborated by the nurse cells, literally pours over the egg, causing a rapid increase in its size. Epithelial cells which surround the nurse chamber above never multiply, but those around the egg multiply rapidly and help to accommodate the protoplasmic substance which pours over the nurse chamber above. Soon a constriction becomes evident at the junction of the two chambers, due partly to the ingrowth of the epithelial cells at the base of the nurse

chamber, and partly to the rapid expansion of the egg and nurse cells. The epithelial cells surrounding the egg chamber are cubical or elongate ovoid in shape, and actively divide, while those surrounding the constriction are smaller and spindle-shaped. No mitosis was observed among the latter.

The brief observation made in the course of the present investigation fully substantiates the findings of Shinji, although he worked upon three species entirely different from *Aonidiella citrina*. It is to his excellent paper that the reader is referred for further cytological and embryological information.

The female reproductive system of *Aonidiella citrina* (fig. 9B) is situated in the body cavity, ventral to the digestive system, and postero-dorsal to the main thoracic ganglion. It consists of a pair of ovaries joined by their respective oviducts to a slender well developed vagina, and forms a figure much the shape of a capital Y. Each ovary carries a large number of ovarioles which are closely and irregularly arranged around the lumen of the oviduct. The ovarioles vary in shape according to their stage of development. In a fully developed ovariole (fig. 9C) two regions can be recognized: the first, a proximal chamber containing a large egg cell with its chorion, protoplasm, yolk, and fat cells; the second, a distal chamber containing only three nutritive cells with their large nuclei, and protoplasm of varying density. Since no division takes place in the epithelial cells surrounding the nurse chamber, this layer is appreciably thinner than the corresponding one around the egg chamber, where, as shown before, active cellular division continues. A so-called nutritive cord, starting from a clear protoplasmic center between the nutritive cells, runs through the narrow connecting neck into the egg cell where it spreads in a fanlike manner. Its function, as the name indicates, is for the conveyance of the nutritive protoplasmic substances, essential for the development of the egg. With the growth of the embryo, the contents of the nurse chamber are withdrawn into the egg chamber, with the result that the epithelial layer of the former shrinks to a small mass. When fully developed, the embryo, enveloped in its amniotic covering, passes into the lumen of the oviduct.

The opening of the *seminal receptacle* is found at the junction of the two oviducts (figs. 9A and 9C). The seminal receptacle is a well developed blind sac which, in the case of fertilized females, is filled with an exceedingly large number of male sperm cells. Located posteriorly of the junction is the vagina, which is a relatively wide canal with its opening on the ventral surface of the body, between the fifth and sixth abdominal segments. Just forward of the opening, the wall of the vagina forms a slight dilatation or uterus. It is probably in the uterus that the

embryo loses its amniotic covering, shortly to appear as a free-moving nymph. Numerous unicellular glands are found around the uterus, while the wall of the vagina itself is of thick intima covered externally by a layer of muscles.

The *male organs* (fig. 10A) vary remarkably in structure according to the different metamorphic stages. In this discussion only those of the adult male will be briefly considered.

Occupying the dorso-lateral space of the body cavity and situated above the alimentary canal is a pair of large, ovoid testes. Each testicular lumen is homogeneously filled with a mass of coiled, threadlike spermatozoa. Leading from the testes are the paired vasa deferentia; the latter are short and thick, each having a lumen surrounded by a well developed epithelium. The vasa deferentia unite with a long and slender ejaculatory duct which, after a few convolutions, opens posteriorly into the style. The ejaculatory duct is provided with a chitinous-walled lumen surrounded by a stratum of epithelial cells, which is followed by a coat of muscular tissue. No vesicula seminalis or accessory glands were found to be present.

*Central Nervous System.*—The central nervous system (fig. 11B) is comparatively well developed for such an inactive insect as *Aonidiella citrina*. It consists mainly of a cephalic ganglion and a thoracic ganglion or thoracico-abdominal center. The latter is the result of an advanced degree of centralization and fusion of the thoracic and the abdominal ganglia, with the further incorporation of the subesophageal ganglion. No ganglia or nerves representing a sympathetic system were found.

The *cephalic ganglion* (figs. 11B and 11C) or brain, situated forward of the mouth region, consists of a bilobed body sending out, from its anterior end, two pairs of nerves: the inner pair representing the ocular nerves, and the outer and shorter pair, the antennal nerves. Of the three cerebral regions, the tritocerebrum, projecting downward, is the only one that is clearly distinguished; the other regions, as a result of the loss of eyes and antennae, are rudimentary and difficult to differentiate. From the supero-posterior part of each brain half arises an esophageal nerve commissure which extends backward, passing on the outside of the esophagus, and continues posteriorly, connecting with the thoracic ganglion (figs. 10D and 11C).

The *thoracic ganglion* (fig. 11A) is a prominent, compressed body, situated in the median portion of the body. It consists, as said before, of five pairs of ganglia fused together, representing the subesophageal ganglion, the three thoracic ganglia, and the abdominal ganglion. The



component thoracic and abdominal ganglia each give off lateral nerves which further divide into smaller nerves supplying the body organs.

Surrounding the medullary "Punkt substanz" (fig. 10D) of the cephalic and thoracic ganglia, there is a well developed cortical layer made up of numerous ganglionic cells. Each ganglionic cell contains a well defined nucleus which, exclusive of the periphery, takes up the haematoxylin stain very rapidly. The ganglia, as well as the nerves, are further enveloped in a thin, transparent membrane or epineurium.

In regard to visible response to external stimuli, a similar condition as noticed by Childs<sup>(3)</sup> in *Epidiaspis*, also held true here:

The only movement that could be noticed was that of the drawing in or telescoping of the posterior region when touched with a needle. This shortening takes place through the contraction of the segments. No other movements of the body were observed in response to other stimulants, such as light, heat, and water.

*Respiratory System.*—*Aonidiella citrina* possesses a respiratory system more or less characteristic of the entire Diaspine group. The whole system was studied with ease by examining early third-stage females under the microscope. Specimens, unattached to their scale coverings, were mounted opposite a small, solid-black area on a microscopic slide, either in water or glycerin. By this method the silvery-white trachea stood out against the black background. The general arrangement of the tracheal system is shown in figure 11B. Only the outstanding trunks and branches are indicated, as the minor branches with their ramifications among the internal organs, form a very complex system, and it would serve no useful purpose to try to represent them here.

The respiratory system (figs. 10B and 10C) consists of two pairs of stigmatic openings or *spiracles*, placed equidistantly on the ventral surface of the body, well within the margin. The anterior pair of spiracles is called the mesothoracic spiracles, and the posterior pair the metathoracic spiracles. The supplementary dorsal abdominal spiracles (mentioned by Newstead<sup>(23)</sup> as being present in *Stigmacoccus* and *Perissopneumon* of the Monophlebinae, and by Oguma<sup>(28)</sup> in *Xylococcus* of the Xylococcinae) do not occur in the Diaspinae. The tracheae are numbered and named, according to the original areas they cover, and to their corresponding homologies in the free-moving nymphs.

Each mesothoracic spiracle forms the opening of a short, wide trunk which immediately branches internally, giving rise to branches *a*, *15*, *b*, *2*, and *c*.

Branch *a* divides, giving rise to the ocular (*16*) and foreleg (*17*) tracheae.

Branch 15 covers the antennal area and becomes forked toward its extremity.

Branch *b* divides, forming the anterior (18) and the posterior (19) tracheae of the mouth region, and also the dorsal anterior trachea (6).

Branch 2 traverses the middle of the body and joins its fellow from the other side, thus forming the mesothoracic transverse tracheal trunk. Before this fusion is made, the longitudinal thoracic tracheal trunk (5), the original middle leg trachea (11), and the ventral thoracic trachea (1), are given off. About halfway down trunk 5, the median lateral trachea (14) branches off.

Branch *c* divides, giving rise to the posterior (12) and the anterior lateral tracheae (13).

Each of the metathoracic spiracles also leads into a short trunk which immediately divides into seven branches. These are:

Branch 5*b* passes anteriorly and unites with branch 5*a* of the mesothoracic spiracle, thus forming the longitudinal thoracic tracheal trunk.

Branch 10 is the original wing trachea.

Branch 9 is the original hind leg trachea.

Branches 8, 3, and 7 form the posterior abdominal tracheae.

Branch 4 (as with branch 2 in the mesothorax) unites with its mate from the other metathoracic spiracle, thus forming the metathoracic transverse tracheal trunk.

The aperture of the spiracle itself is surrounded by a chitinous rim (fig. 10*C*) which is continuous ventrally with what is known as the muscle plate. The opening leads into a chamber called the collar chamber, which extends on each side, forming two small lobes. At the base of the chamber, ridgelike processes were noticed projecting into the lumen. These projections, together with muscles running to the muscle plate, probably constitute a closing apparatus, similar to that which was shown by Savage<sup>(29)</sup> to be very well developed in *Monophlebus* (Monophlebinae). Although longitudinal and cross sections of the tracheal trunks were examined, in no instance was the presence of taenidial rings detected.

*Dorsal Vessel*.—List,<sup>(18)</sup> working on the internal anatomy of *Orthezia cataphracta*, is perhaps the only worker who has definitely described a dorsal vessel for a member of the Coccidae. He writes:

Bei Herauspräparation des Darmes und der Malpighi'schen Gefäße gelang es mir, einen aus zarten Wänden bestehenden Schlauch, der an der äußeren Membranen der Malpighi'schen Gefäße haftete, zu beobachten. Er lag auf der dorsalen Seite derselben, hatte eine bedeutende Länge und musste, so viel ich aus derselben schätzen konnte, bis in der vorderen Theil des Thorax reichen.

The presence of such a complete organ of circulation in the Coccidae has subsequently been doubted by Oguma<sup>(2b)</sup> and other investigators, with the result that at present the Coccidae are quoted as not possessing an organ comparable to the dorsal vessel in other insects. Blood circulation in this group is described as taking place by the contraction and expansion of certain body cavities, especially the pericardial sinus.

After a thorough study was made of all the internal organs, the writer became fully convinced that *Aonidiella citrina* does possess a dorsal vessel (fig. 12A). A highly nucleated duct is found running from the caudal extremity of the body, through the thorax, and into the head where it terminates in the region of the cephalic ganglion. It follows throughout the median dorsal line just beneath the integument. Longitudinal and cross sections through the stomach show the dorsal vessel to be in close association with the outer wall of the latter. Posterior to this portion it widens appreciably to form the heart, after which it tapers down to a fine tube toward both the abdominal and cephalic regions.

Because of the delicate nature and small size of the dorsal vessel, not much could be learned about the details of its structure, or of the absence or presence of ostia and aortic valves. However, its walls are decidedly cellular, the cells showing a large number of nuclei regularly arranged around a fairly distinct lumen which is further surrounded by a more or less granular protoplasm. The cells are covered externally by a muscular layer, the fibers of which are extremely hard to detect.

Additional evidence as to the presence of a dorsal vessel was brought out by the direct examination of live specimens under the microscope. Studied from a dorsal aspect, a steady and regular pulsation was noticed dorsad of the stomach and at the same position that the dorsal vessel was located in the stained sections.

*Glands.*—The glands of *Aonidiella citrina* are of four types: salivary, wax, pygidial, and esophageal.

The *salivary glands* (fig. 12B) are paired, tubular, lobelike structures situated on each side of the fore intestine, and occupying practically the whole of the lateral parts of the mesothorax. Each gland consists of numerous glandular cells arranged radially around branched ducts running from the lobelike structures. The cells or acini are surrounded by a thick layer of granular cytoplasm in which are imbedded numerous large nuclei. The branched ducts open into a common median duct which pursues an inward and forward course. When in close proximity to the mouth region, this median duct turns inward, uniting with another from the opposite corresponding gland, to form a common outlet, opening into the side of the pumping apparatus. Throughout its whole

length, the duct exhibits a narrow but distinct lumen which is lined with a thick, chitinous intima.

Because of their variation in form, size, and location, the *wax glands* (fig. 12D) play an important part in the classification of the Coccidae. In the generalized or unarmored coccids, like *Lecanium*, *Xylococcus*, or *Orthezia*, the openings of these glands are scattered over the whole cuticula of the body. In the specialized or armored coccids, the openings are situated in more definite groups toward the posterior end. They may be limited to the lateral halves of the pygidium, where their openings are more or less arranged in three marginal lines on the dorsum (figs. 2A and 2C), as in *Aonidiella citrina*, or an additional group may be found around the genital opening, the latter being known as the circum-genital glands.

Figure 12D shows the structure of one of these wax glands. Each opening forms the outlet to an extremely long and slender invaginated tube, known as the ceratuba. At its anterior extremity, each ceratuba is connected to a highly chitinized, perforated, knoblike structure, which forms a reservoir for the reception of the substances secreted by the central wax gland and the two lateral or accessory glands. The central wax gland consists of an anterior apical, and a posterior cylindrical region, the two being connected by a relatively short stem or neck. Both these regions stand in conjunction with a narrow duct, which, running posteriorly, opens into the chitinous reservoir. The apical region is typically glandular in structure, containing a large nucleus; the posterior cylindrical region, in addition to being glandular, possesses a layer of cells arranged around the common duct, similar to those found in the salivary glands. The paired, unicellular accessory glands which open into the chitinous chamber, on each side of the wax gland opening, differ somewhat from the latter, both in shape and structure. They are more ovoidal and each contains a smaller, though clearly visible, nucleus toward the distal end. Their protoplasmic masses, moreover, are more homogeneous throughout, hardly showing any vacuoles or glandular intrusions. The outstanding difference, however, is their peculiar lumens which, as a result of five or six projecting processes, take on a branchlike appearance. Each lumen tapers posteriorly to form a short, excretory duct which opens into the reservoir.

According to Berlese and Leonardi,<sup>(1)</sup> the accessory glands secrete a varnishlike substance, which forms a protective layer around the wax thread as the latter is produced by the central wax gland. This theory is quite plausible, as the hard texture and the polished appearance of the female scale covering is probably due to this varnish.

The *pygidial glands* (fig. 12C), so called because of their similarity to those of other insects, are paired organs situated posteriorly on each side of the hind intestine. Their respective ducts unite to open shortly in close association with the anus. Each gland is composed of many multicellular lobelike bodies surrounding a poorly defined internal duct. The function of the pygidial glands is not known, but it is very probable that they are also for wax production.

The *esophageal glands*, as pointed out in connection with the structure of the esophagus, are present as small, glandular cells imbedded in the epithelium of the latter.

### SUMMARY

The yellow scale, *Aonidiella citrina* (Coq.), is here recognized as a distinct species and not as a variety of the red scale, *Aonidiella aurantii* (Mask.). This segregation is made as a result of evidence based upon a comparative study of their ecology, biology, and morphology.

Although not well defined in their distribution, the yellow scale seems to prefer the warmer valleys and foothills of the interior, while the red is more abundant closer to the coast.

The red scale attacks all parts of the host tree, whereas the yellow is limited almost entirely to the leaves and fruit.

Reared under similar conditions, the yellow scale completes its life cycle in about 65 days and the red scale in about 60 days, giving an approximate difference of 5 days.

Structural differences are noticed in the pygidial fringes of the two scales. Apart from possessing slenderer lobes and minor variations in the median plates, the pygidium of the yellow scale shows the presence of a fourth lobelike process. Differences in color and texture of the scale covering are also noticed.

In addition to a comparative study of the two species a study was made of the anatomy of *Aonidiella citrina*, which is summarized herewith.

The digestive system is relatively short and straight, with the esophagus and ventriculus showing the highest degree of development. In opposition to Berlese's finding, a definite connection is found between the ventriculus and the small intestine. The gastric epithelium consists of large cells which do not project into the lumen of the ventriculus.

The female reproductive system consists of two well developed ovaries connected to each other in the usual Y-shaped manner. Each ovary carries numerous ovarioles in different stages of development. A fairly large seminal receptacle occurs at the point of fusion of the two ovaries.

The male organs are of the usual type, namely, a pair of testes opening into a long style or penis.

The central nervous system is well defined and consists of two main regions, namely, the cephalic and the thoracic ganglia, the latter having been formed as a result of the fusion of the component subesophageal, the thoracic, and the abdominal ganglia. Antennal, ocular, and lateral nerves are found.

The respiratory system is composed of well developed tracheae which ramify through the whole body. Two spiracles are found on the median-ventral surface of the body. The tracheal branches are classified according to their position.

A profusely nucleated dorsal vessel, consisting of a heart and aorta, runs practically the length of the body just below the dorsal surface. A definite pulsation is noticed.

Salivary, wax, pygidial, and esophageal glands are discussed.

#### ACKNOWLEDGMENTS

Acknowledgement is due Professor H. J. Quayle, who suggested this problem and under whose direction the biological studies were made from June, 1928, to June, 1929; to Professor O. A. Johannsen of Cornell University, for his kind assistance and encouragement in details of the internal anatomy; to Professor G. W. Herrick of Cornell University, for valuable suggestions; and to A. M. Boyce, who was kind enough to furnish me, from time to time, with fresh material to be used in the anatomical part of this study.

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## ABBREVIATIONS USED IN THE FIGURES

abd.g.	abdominal ganglion	f.ep.	follicular epithelium	p. eye	primary eye
ac.gl.	accessory gland	f.leg	fore leg	pha.	pharynx
a.ch.	antechamber			pln.	plunger
ant.	antenna	gen.hist.	genital histoblast	p.th.	prothorax
ant.hist.	antennal histoblast	ger c.	germ cells	pu.s.	Punkt substans
ant.n.	antennal nerve	gl c.	gland cells	pyg.gld..	pygidial gland
ar.inf.	arcus inferior	gl.du.	glandular duct		
ar.sup.	arcus superior	gl.o.	gland opening		
		gr.	groove	r.	retina
b.sut.	basal suture	ha.	halter	rect.	rectum
bu.fr.	buccal frame	h.leg	hind leg	ret.mascl.	retractro muscles
bu.mascl.	buccal muscles	ht.	heart	ros.	rostrum
		hyp.	hypodermis	rstis.	rostralis
cae.	caecum				
ca.v	cardial valve	i.	iris	sal.gld.	salivary gland
c.ch.	collar chamber	int.sc.	interscutellar band	sc.	scape
ceph.g.	cephalic ganglion			scl.	scutellum
ceph.r.	cephalic ridge	l.	lens	scu	scutum
cet.	ceratuba	L.	lobe	sp.	spermatozoa
c.gl.	central gland	l.hist.	leg histoblast	spi.	spiracle
ch.l.	chitinous layer	ln.	lateral nerve	s.rec.	seminal receptacle
ch r.	chitinous ridge	l st.	linear sternite	st.	stem or neck
c.hyp.	corneal hypodermis	lu.	lumen	sty.	style or penis
cla.	clavus			subes.g	subesophageal gang-
c ne	connecting neck	mal t.	Malpighian tube		lion
co.inf.	costa inferior	ma r.	malar ridge	te.	testis
co.l.	cortical layer	mal.b	Malpighian body (bulb)	th g.	thoracic ganglion
con.	connection	m cav.	mouth cavity	Tr	triangular lobelike
co.sup.	costa superior	m.du.	median duct		process
c.pls.	chitinous plates	mes.p.	mesothoracic promi-	tra.	trachea
cru.	crumena		nence	tri c.	tritocerebrum
cs.	conus	mes.sp	mesothoracic spiracle	tro.	trochanter
cyl.	cylinder	met sp.	metathoracic spiracle		
cyt.	granular cytoplasm	m.leg	middle leg	u.gl.	unicellular glands
		m pl	muscle plate	ut.	uterus
d.ac. eye	dorsal accessory eye	mascl.	muscles	uv.	uva
div mascl.	divaricator muscles	m.st.	mesosternum		
dor.v.	dorsal vessel	m th.	metathorax	v.ac. eye	ventral accessory eye
du.	duct	m.tub.	mouth tubercle	vag	vagina
				v def.	vasa deferentia
e.cl.	egg cell	n.cl.	nurse cell	ventr.	ventriculus
e.du.	ejaculatory duct	nu	nucleus	vi.r.	visual rods
ep.	epimera	nu.c.	nutritive cord		
epi.	epineurium				
es.	esophagus	oc ap.	ocular apophysis		
es.com.	esophageal com-	oc n.	ocular nerve	wg.	wing
	missures	o du	oviduct	w.hist.	wing histoblast
es.gl.	esophageal gl	op n.	optic nerve		

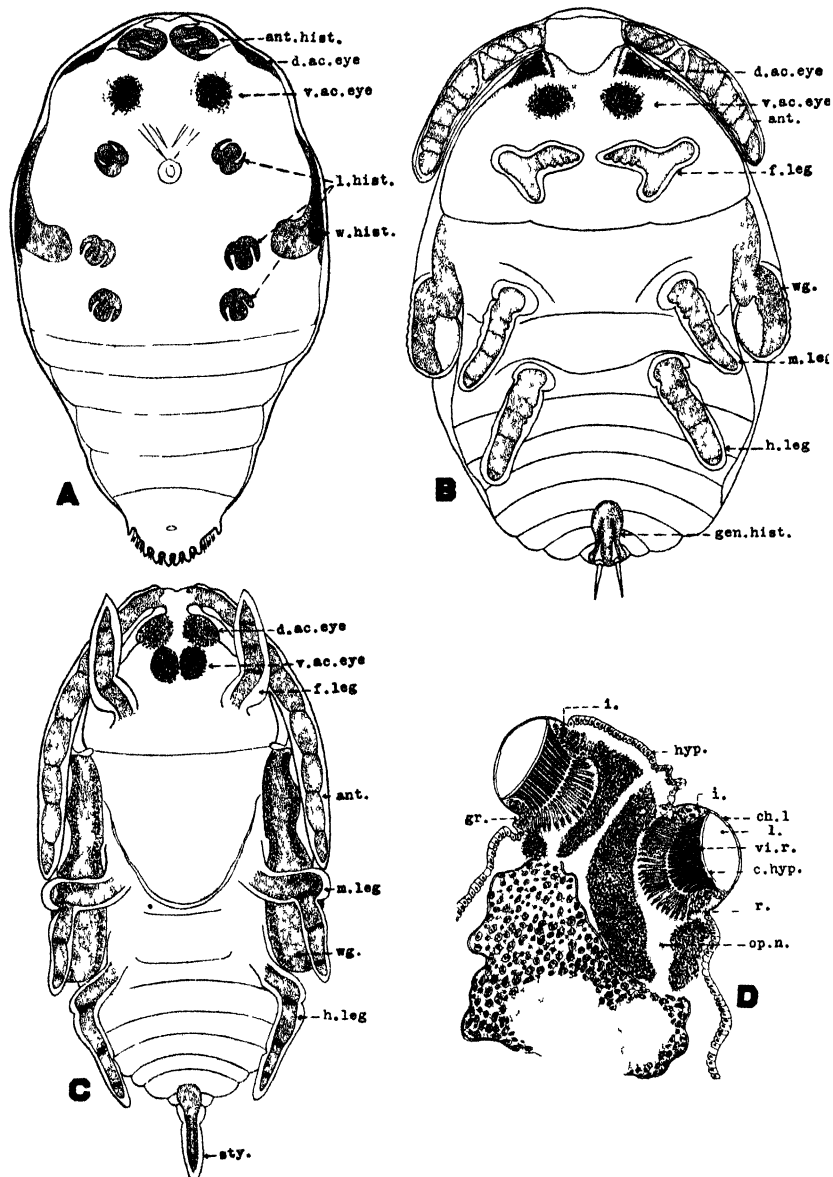


Fig. 1. Metamorphosis of the male. *A*, second stage; *B*, prepupa, *C*, pupa; *D*, longitudinal section through head of an adult male showing structures of dorsal and ventral accessory eyes. Explanation of abbreviations used in this and succeeding figures will be found on page 456.

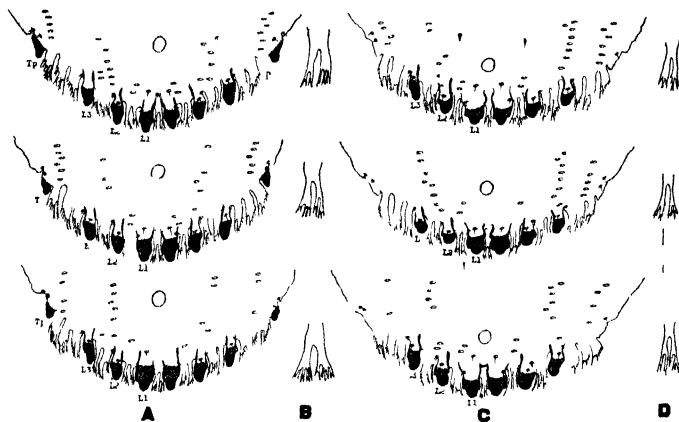


Fig 2 *A*, pygidia of yellow scale, showing fourth lobelike process, *B*, median plates of yellow scale, *C*, pygidia of red scale, *D*, median plates of red scale

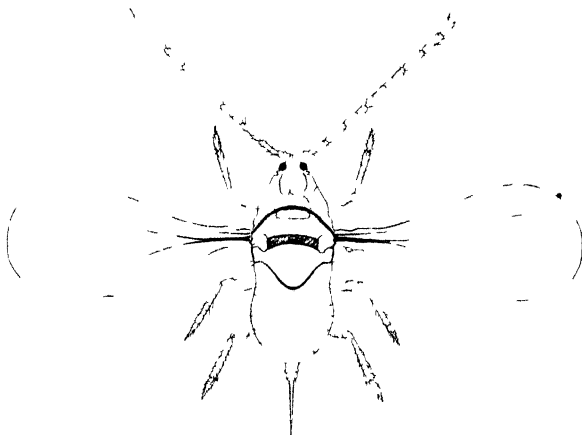


Fig 3 Adult male of *Aonidulla citrina* ( $\times 47$ )

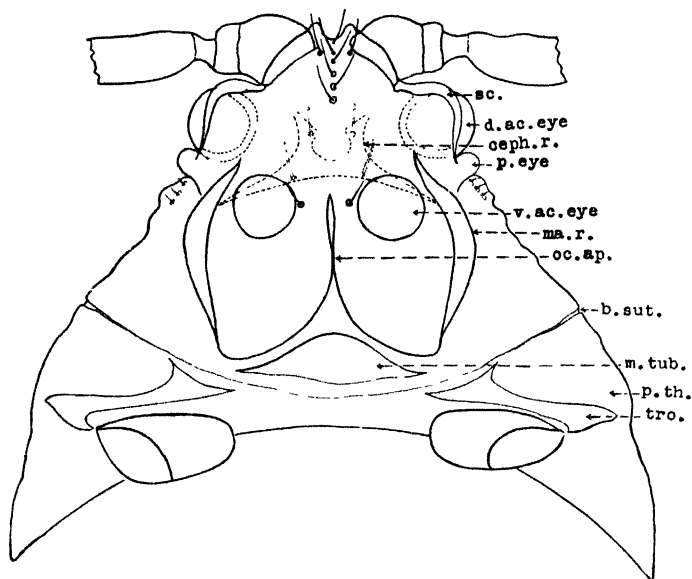


Fig. 4. Ventral view of head of adult male of *Aonidiella citrina*.

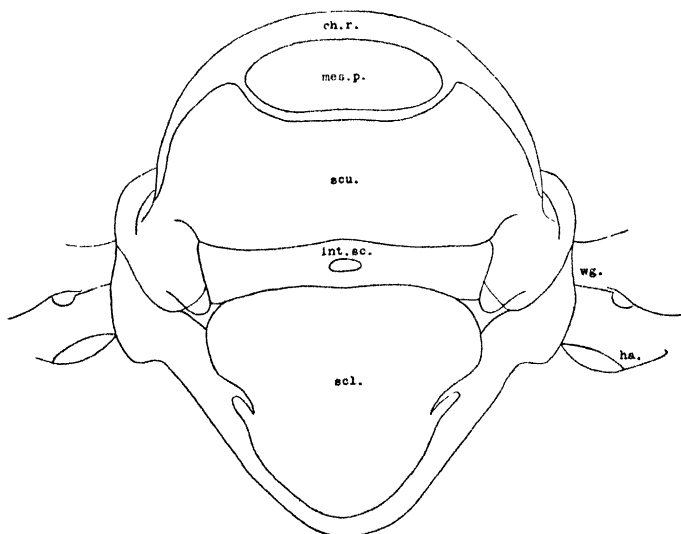


Fig. 5. Dorsal view of mesothorax of adult male of *Aonidiella citrina*.

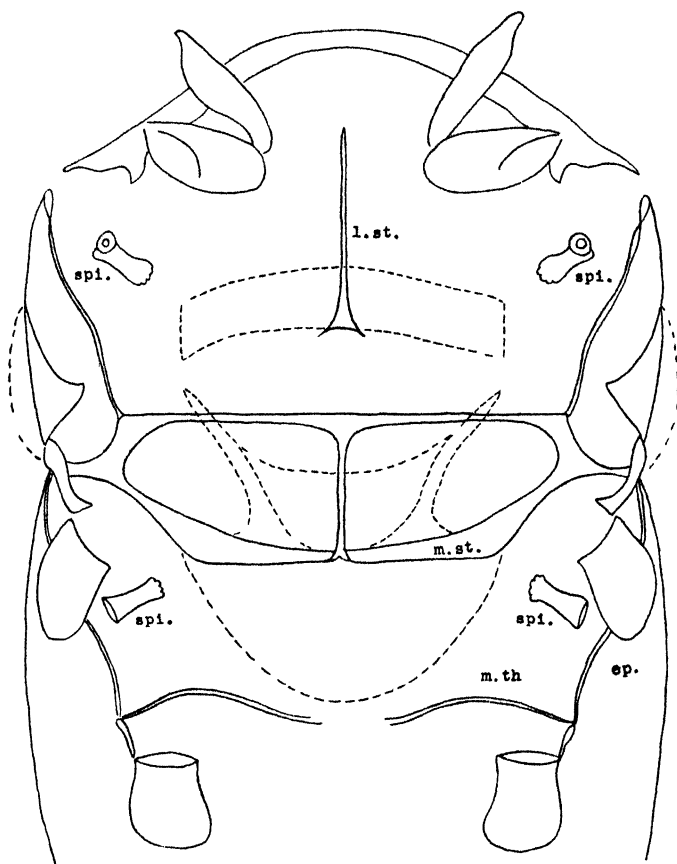


Fig 6 Ventral view of mesothorax and metathorax of adult male of *Aonidiella citrina*.

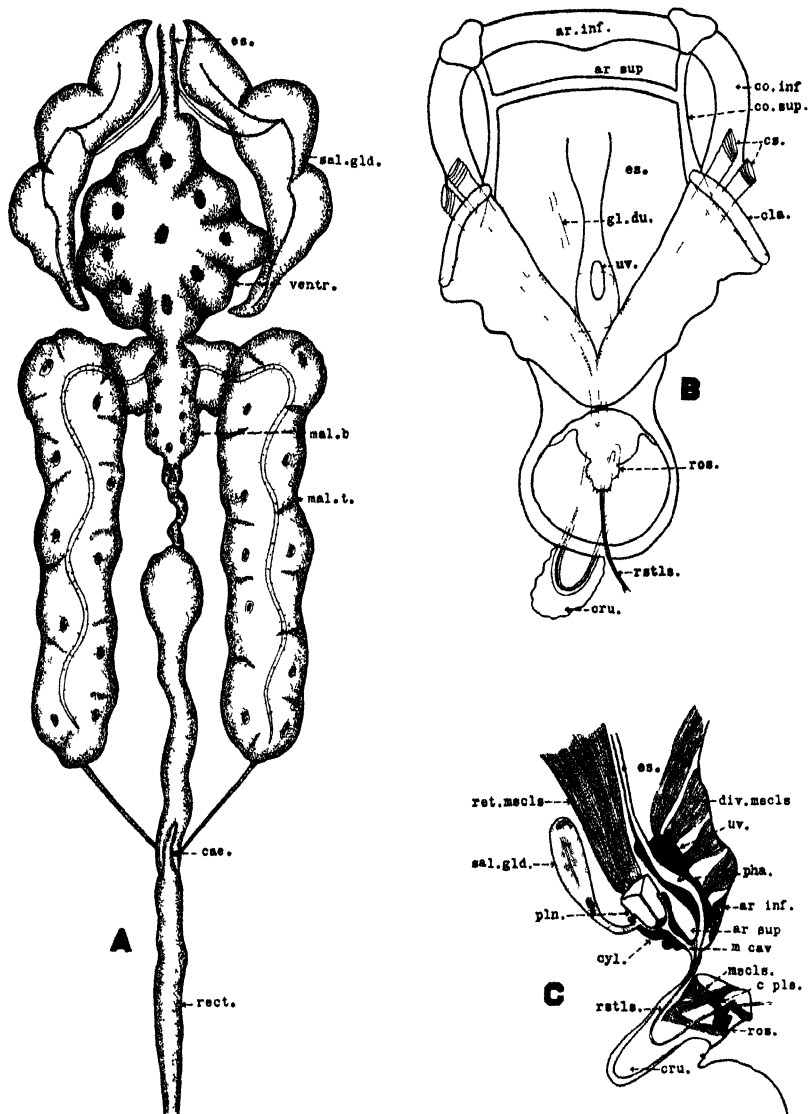


Fig 7. A, digestive system of *Aonidiella citrina*; B, buccal frame (diagrammatic); C, longitudinal section of mouth parts

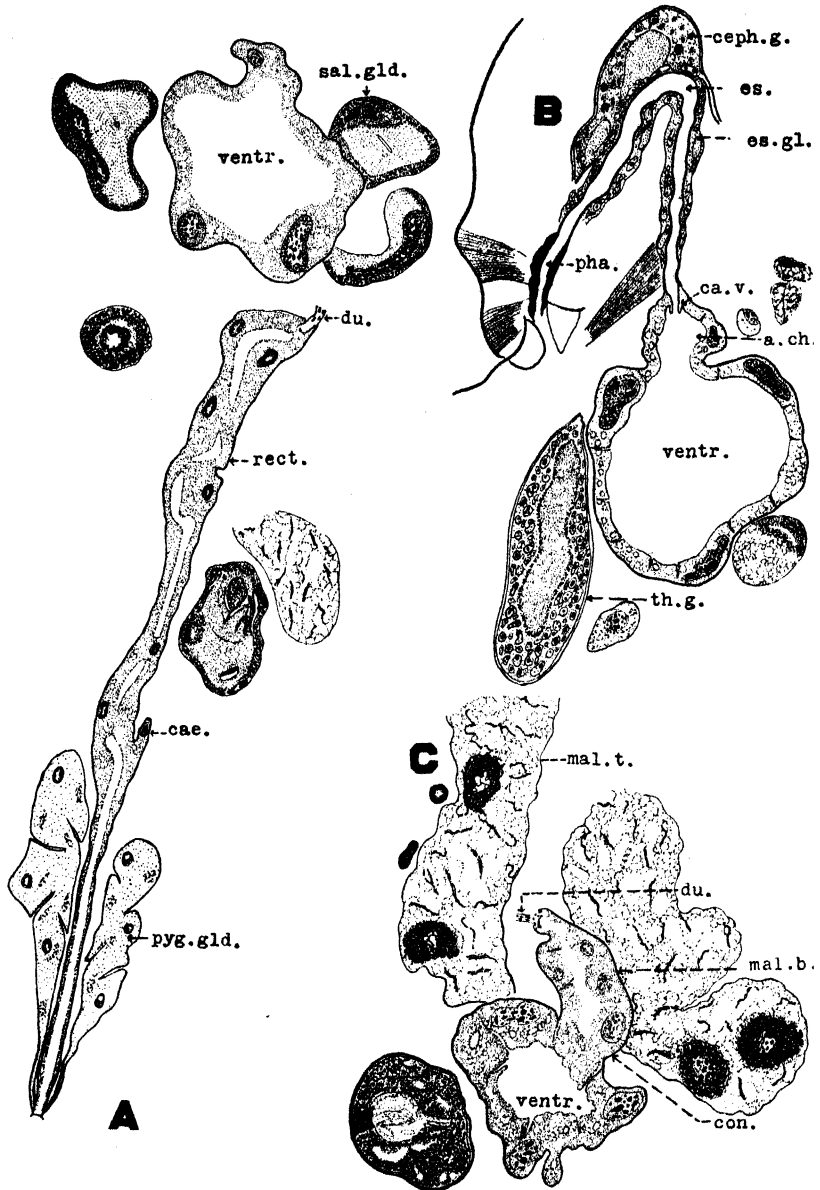


Fig. 8. *A*, horizontal section showing hind intestine; *B*, longitudinal section showing esophagus and ventriculus; *C*, horizontal section showing connection between ventriculus and Malpighian body.

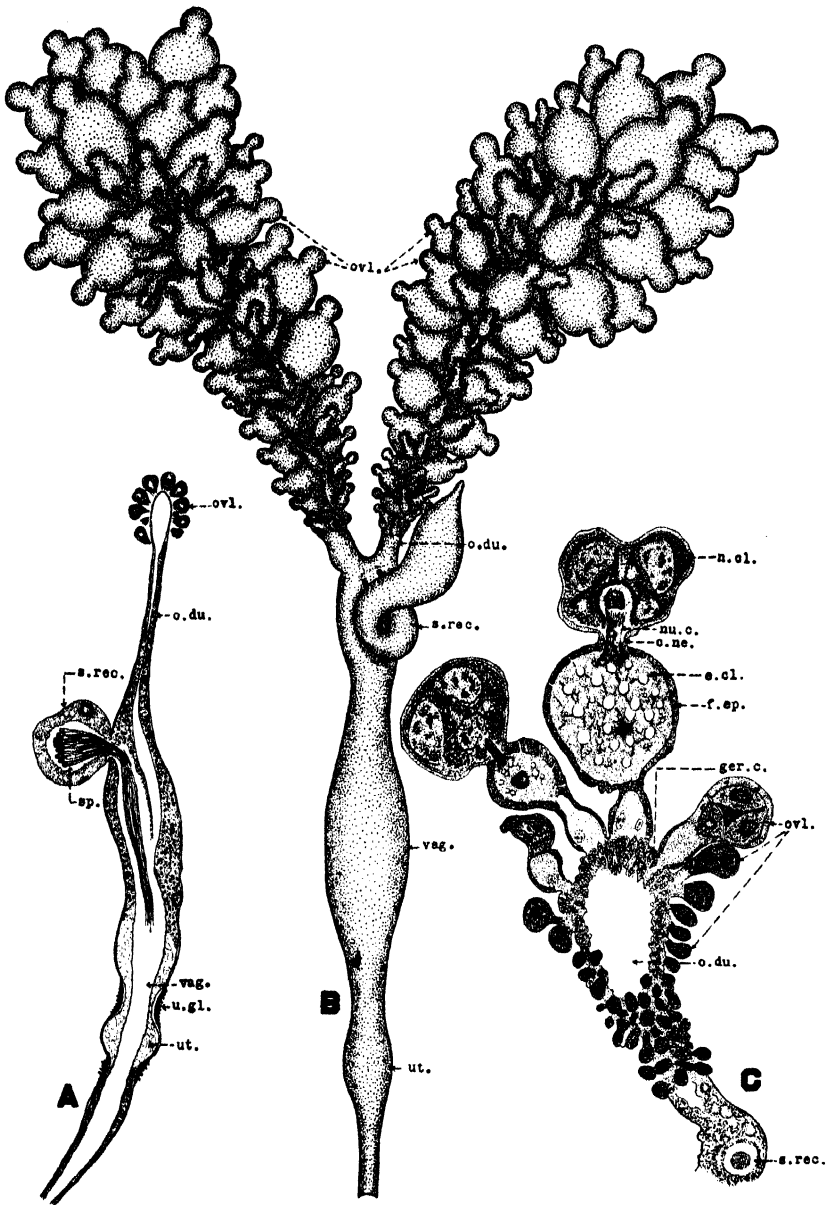


Fig. 9. *A*, longitudinal section of oviduct of *Aonidiella citrina*; *B*, female reproductive system; *C*, horizontal section through an ovary.



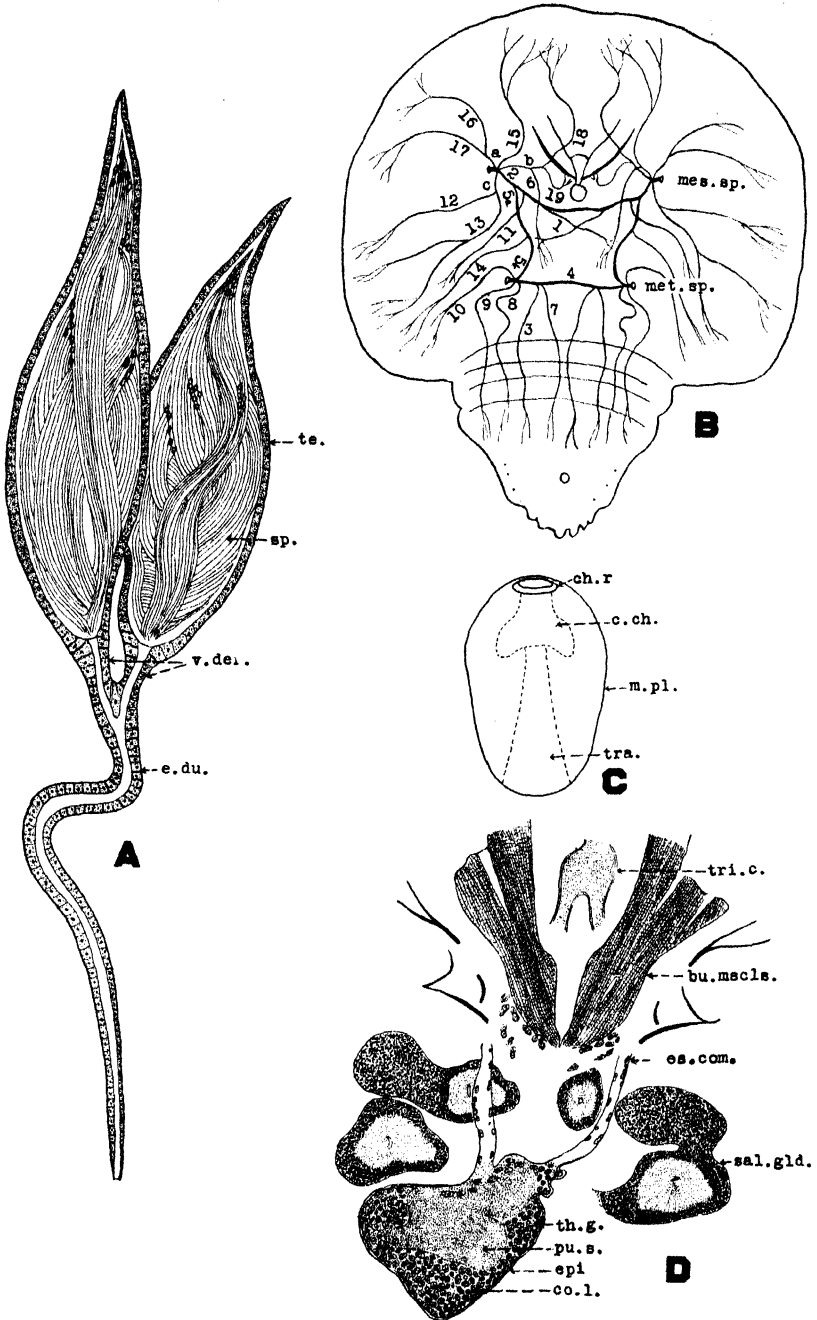


Fig. 10. *A*, male reproductive system of *Aonidiella citrina*; *B*, respiratory system (semidiagrammatic); *C*, diagrammatic sketch of a spiracle; *D*, horizontal section showing esophageal commissures connecting with thoracic ganglion.

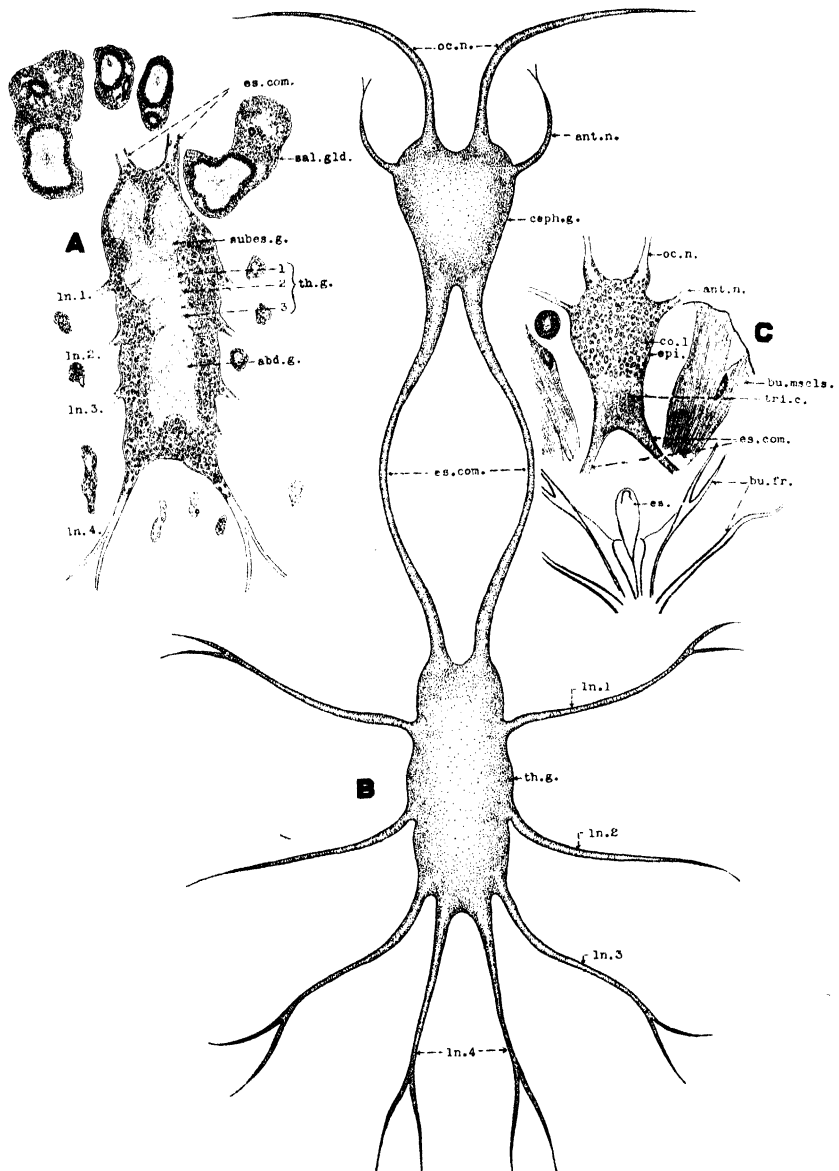


Fig. 11. *A*, horizontal section through thoracic ganglion, showing fusion of subesophageal, thoracic, and abdominal ganglia; central nervous system; *C*, horizontal section through cephalic ganglion.

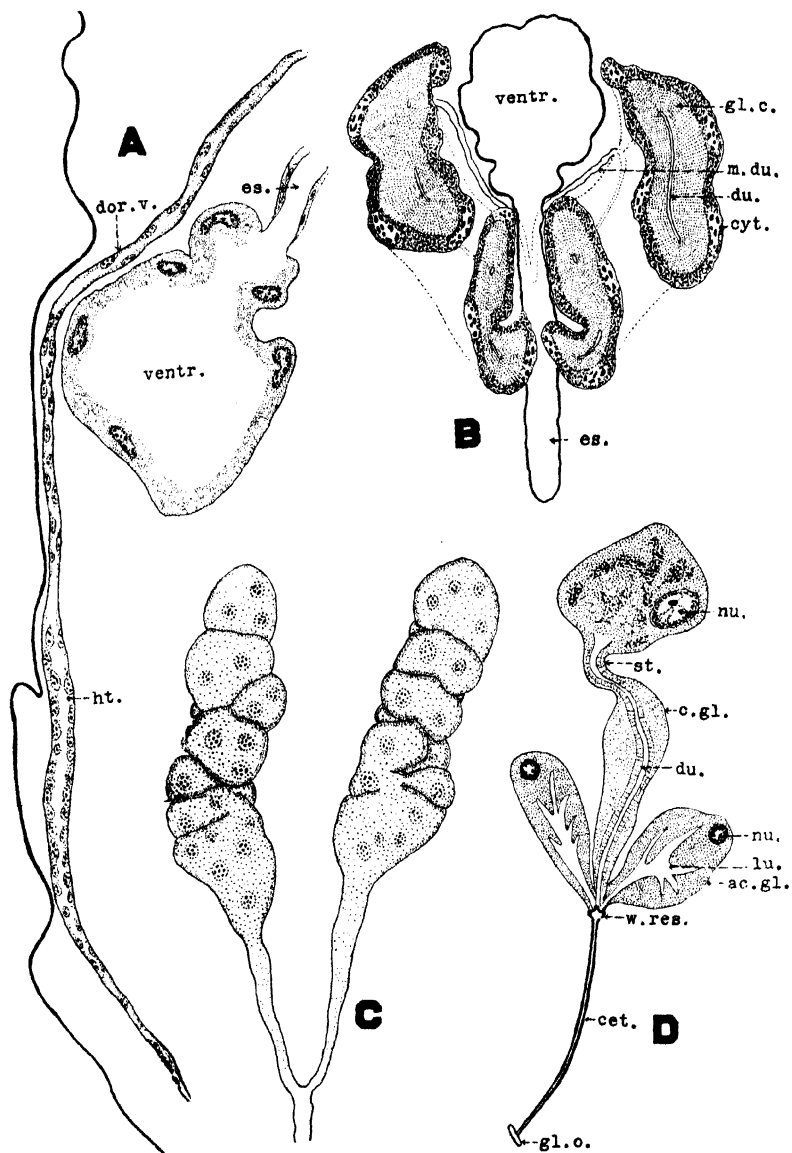


Fig. 12. *A*, longitudinal section showing dorsal vessel and heart; *B*, horizontal section showing salivary glands; *C*, pygidial glands; *D*, a wax gland.

# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

JUNE, 1933

No. 12

## THE BIOLOGY OF THE BEAN THRIPS<sup>1</sup>

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Despite the great amount of research being done in the various orders, a study of the biology of the insects belonging to the order Thysanoptera still offers a fertile field. The life histories of many of these minute insects are still unknown; also a comparison of their various habits and activities presents extremely interesting material for speculation, emphasizing the need for detailed work of an exhaustive nature before the phylogeny of the group can be clarified. Doubtless the small size and elusive habit of the thrips, in addition to difficulties encountered in rearing and handling them, have been obstacles to the study of this group.

The bean thrips has become, in recent years, an increasingly important pest of several commercial crops, particularly in the dry interior valleys and nonirrigated sections of California. The problem presents several very interesting phases of an ecological nature and it is the two-fold purpose of this paper to make the relationships of the group more perspicuous and, while not treating directly of control measures, to present information upon which a basis for future control might be established.

### SYNONYMY AND DESCRIPTION

*Synonymy.*—Fortunately, perhaps, in contrast with many important insect pests, the history of the bean thrips is not very long nor the synonymy highly involved.

The first record of this thrips seems to be in 1895 when it was reported as collected in Yuba County, California, in November, 1894. Mr. G. W. Harney collected two specimens at this time on an orange leaf. It was on one of these specimens that Theodor Pergande's original description (Pergande, 1895) was based.

<sup>1</sup> Received for publication July 13, 1932.

<sup>2</sup> Junior Entomologist in the Experiment Station.

No further record of the bean thrips is found until 1902 when Hinds redescribed this species from the one female specimen deposited in the Division of Entomology in Washington, D. C., by Mr. Harney in 1894. The male was not known and no knowledge of the life history was to be had at this time. Hinds (1902) changed the specific name from *fasciata* of Pergande to *fasciatus*, as he stated:

"Thrips is a Latin name derived from the Greek, meaning a wood-louse, and is in the singular number and masculine gender, as will be also all generic names of which it forms the termination."

Miss S. M. Daniel (1904) recorded five new species of Thysanoptera from California, in addition to *Frankliniella occidentalis* (Perg.), *F. tritici* (Fitch), *Thrips tabaci* Lind., and *Heliothrips fasciatus* Perg., which had already been reported from this state. Among the new species was *Caliothrips woodworthi* which was fully described. However, *Caliothrips woodworthi* was made synonymous with *Heliothrips fasciatus* Perg. by Moulton (1907).

A brief description of the bean thrips was given by D. L. Crawford (1909) in the *Pomona Journal of Entomology*.

O. E. Bremner, in 1910, listed the bean thrips under an incorrect genus as *Euthrips fasciatus*.

A new genus, *Hercothrips*, was erected by J. D. Hood (1927) who made *Heliothrips striatus* Hood the genotype. Sixteen species in all were removed from the genus *Heliothrips* and placed in the new genus *Hercothrips*. *Heliothrips fasciatus* Pergande was among those removed and so it is now synonymous with *Hercothrips fasciatus* (Perg.).

*Description of the Bean Thrips.*—The order *Thysanoptera* is divided into two suborders, the Terebrantia and the Tubulifera; the former has a sawlike ovipositor, while in the latter the terminal abdominal segment is tubelike with no ovipositor. *Hercothrips fasciatus* (Perg.) belongs to the suborder Terebrantia and the family Thripidae on the basis of such characters as down-turned ovipositor, 8-segmented antennae, and conical formation of the last abdominal segment of the female. And, further, following the classification of J. R. Watson (1923), *H. fasciatus* was placed in the genus *Heliothrips* since the wings are fully developed, the fore wings being without knoblike bristles, and the second segment of the antennal style being much longer than the first. However, Hood (1927) placed *fasciatus* in the genus *Hercothrips* on the basis of the costal fringe of bristles on the wings, the forked trichomes of the antennal segments, and the closely approximated hind coxae.

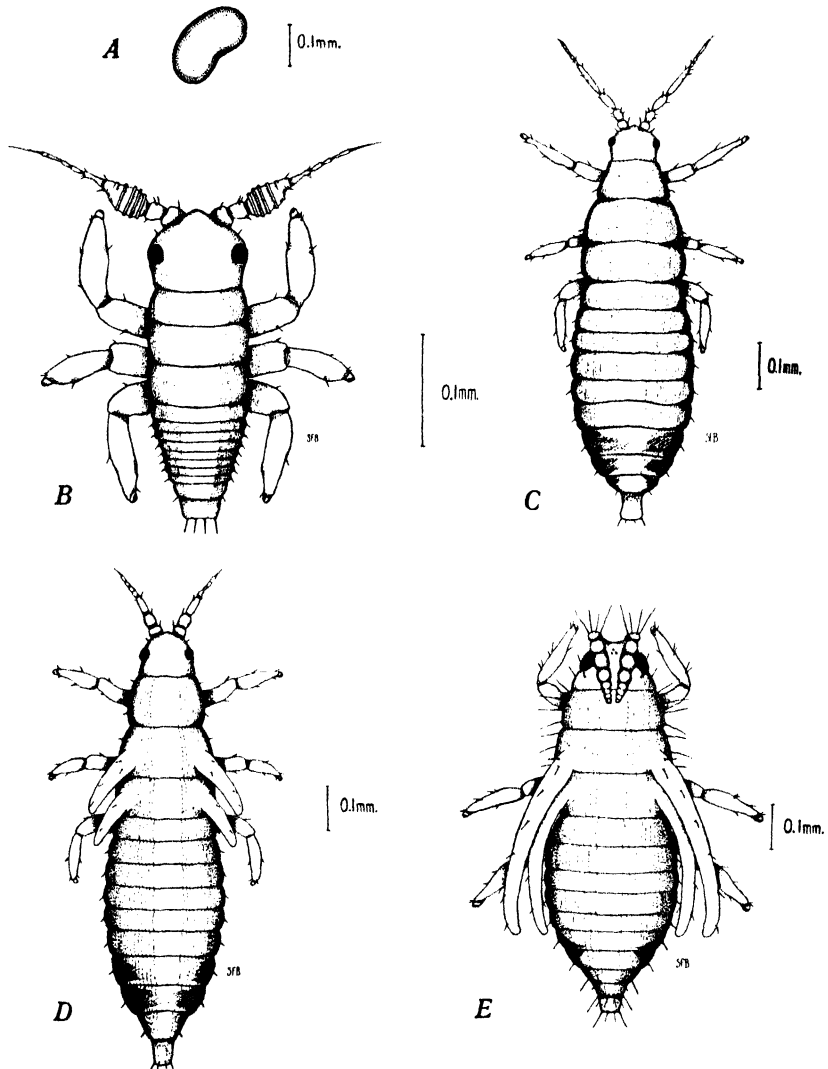


Fig. 1. The bean thrips. *A*, egg; *B*, newly emerged larva; *C*, mature larva; *D*, prepupa; *E*, pupa.

*Egg*.—Very delicate with thin chorion, bean-shaped (fig. 1, *A*), translucent white in color, and smooth; average width or diameter, 0.112 mm; average length, 0.225 mm. With the development of the embryo the egg swells and often the outlines of the larva may be seen, especially the eye-spots.

*Newly Emerged Larva*.—General shape somewhat fusiform; color, translucent white; head, antennae, and legs very large in proportion to body (fig. 1, *B*); average total length, 0.287 mm. Antenna 8-segmented with long style, approximate length, 0.161 mm; third segment very large with rather distinct rings. Eyes blackish red;

ocelli wanting. Setae present, sparse on legs, antennae, and sides of abdomen; longest on posterior abdominal segment. Abdomen tapering, tenth segment very large in proportion and usually upturned.

*Mature or Second-Stage Larva.*—Shape fusiform; reddish yellow in color with variable crimson blotches and bands along sides of thorax and abdomen (fig. 1, C); antennae, legs, and posterior abdominal segment, pale yellow. Setae sparsely scattered on legs, antennae, and margins of thorax and abdomen, becoming larger at sides and on tenth abdominal segment. Average total length, 0.960 mm. Antennae 8-segmented and about 0.256 mm in length. Eyes small, reddish orange; ocelli wanting. Abdomen fusiform, crimson bands usually extending across segments seven, eight, and nine, but considerable variation present in individuals; posterior segment tubular, usually curving upwards.

*Prepupa.*—Shape similar to that of mature larva; color, orange with variable crimson markings at thorax and abdomen, bands of crimson usually extending across seventh and eighth abdominal segments; legs and antennae, translucent white; setae occur sparsely on antennae, legs, and at sides of thorax and abdomen, tenth abdominal segment having ring of setae on posterior margin. Average total length, 1.008 mm. Eyes dark orange with a few facets visible; ocelli wanting. Antenna 8-segmented, length approximately 0.175 mm; segments difficult to distinguish and with much less shape than in larva. Wing pads very short (fig. 1, D), translucent white, with a few scattered setae, fore wing pad extending to first abdominal segment, hind wing pad extending to second abdominal segment. Abdomen fusiform. Sexes extremely difficult to distinguish, females usually somewhat larger and more plump.

*Pupa.*—The pupal stage might well be divided into the early and late stages. In the early stage the general color is orange and the red markings on the sides of the abdomen are smaller and less distinct. The entire body appears much shorter and stouter than the prepupa. Average total length, 0.864 mm; antennae rather shapeless and folded back over head (fig. 1, E), usually bending between first and second segments; segments indistinct and second segment with four long setae projecting forward; length of antennae about 0.162 mm. Eyes much larger and darker red in color than in prepupa; three orange-colored ocelli are plainly visible between eyes in a triangle. Wing pads, like antennae and legs, translucent white; wing pads have scattered setae present on surface and extend to sixth or seventh abdominal segment. Abdomen fusiform in shape with irregularly distributed crimson markings at sides. Setae present along sides of thorax and abdomen, ninth and tenth abdominal segments having long setae on posterior margins. Ovipositor rather clearly demarked in female so that sexes can be readily distinguished.

In the late pupal stage the antennae come forward slowly, straighten out, and begin to darken up noticeably. The abdomen also begins to exhibit considerable pigmentation, particularly along the sides, nearly blotting out the red markings. The wings within their cases next darken and the cross-bands can be distinctly seen. Next in order the legs darken slightly, followed by the head and thorax and the reticulation can be seen on the surface of the body. Molting and the emergence of the adult occurs at about this point.

*Adult Female.*—General shape fusiform; average total length, 1.136 mm; uniform dark brown in color; reticulation faint on head, prothorax, and at sides of meta-

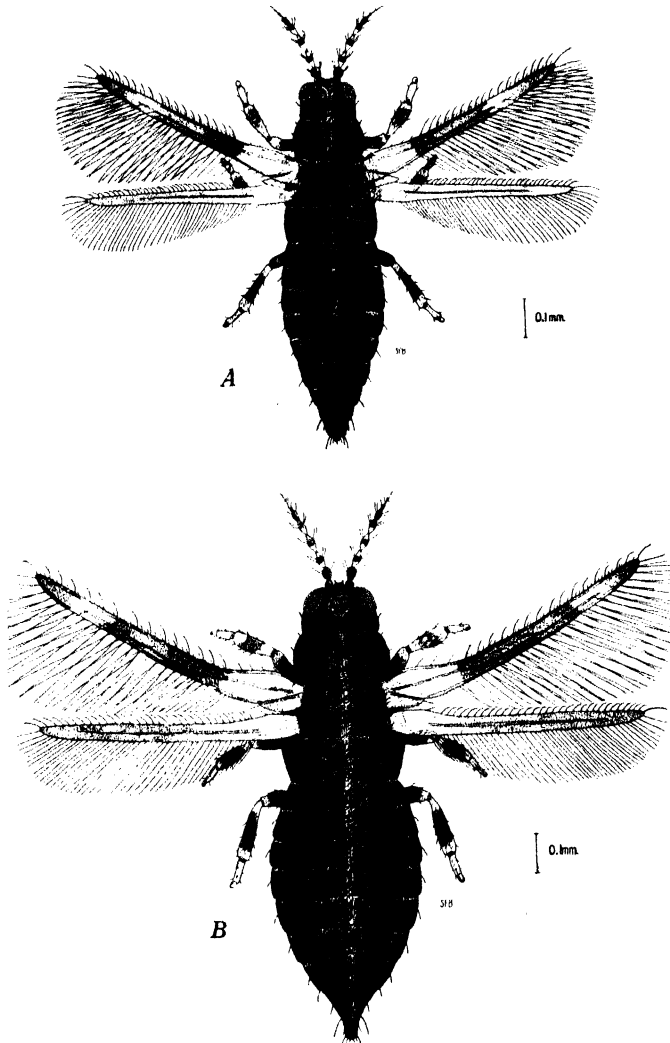


Fig. 2. The bean thrips. *A*, adult male; *B*, adult female.

thorax and abdomen. Head extends forward between antennae in a U-shaped process to about half the length of first antennal segment. Eyes small, black, with facets distinct; ocelli three, in a slightly elevated area between eyes, pale yellowish margined with dark orange. Antennae about 0.265 mm in length, 8-segmented; first and second segments uniform brown, third and fourth with middle third brown and remainder of segments pale yellow; anterior half of fifth segment brown and remainder of segments uniform brown.

Both pairs of wings appear to be joined to the mesothorax (fig. 2, *B*), but the muscular attachments of the hind pair are in the metathorax. Average length of fore



wing, 0.784 mm; average length of hind wing, 0.740 mm; fore wing with one branched vein in the center of wing dividing at inner edge of dark band. Anterior branch becomes contiguous with costal vein and posterior branch continues distinct to center of white area near tip. Costal spines usually 20; number of spines on posterior fork of midvein variable, 5 to 7; number of hairs in fringe on hind margin of wing variable, usually 30. Fore wing grayish brown, darkest along veins; dark area in center of wing equal to about one-half of wing's length, basal fourth white, and distal fourth equally divided into light and dark areas, tip dark. Hind wing uniformly grayish brown, basal fourth light; one longitudinal vein in center of wing becoming indistinct near base and not reaching tip; spines on costal margin about 32 in number; number of hairs in fringe variable, usually 42; entire surface of wings covered with very fine hairs.

Femora dark brown except at tips; tibiae with central portion dark brown, extremities yellow; fore tibiae somewhat lighter brown; setae distributed irregularly on tibiae and tarsi; hind femora only with setae; spines present also on lateral margins of head, prothorax, and abdomen; ninth and tenth segments of abdomen with a row of long spines on posterior margins. Short, strong spines are present on posterior-lateral margins of abdominal segments. Abdomen slightly broader than thorax, ovate, tapering sharply to last segment. Ovipositor visible under magnification, extending up into eighth segment. Approximate length of ovipositor, 0.167 mm.

*Adult Male*.—The male differs but slightly from the female; it is somewhat smaller in size, the abdomen tapers more gradually, and the terminal abdominal segment is more blunt (fig. 2, A). The coloration, markings, and setae are practically identical. Under magnification the reddish-orange testes may be seen suspended in the seventh and eighth abdominal segments. The external portions of the genitalia may be seen to extend slightly beyond the end of the abdomen.

There is another character that readily separates the sexes, as is the case in most of the Terebrantia, i. e., on the ventral surface of the abdominal segments of the male there are transverse elliptical areas, more or less transparent and pale yellow in color. In certain species there are six of these elliptical areas, on the second to the seventh segments inclusive. However, in *Hercothrips fasciatus* there are only five of these areas present on the second to the sixth segments; the female does not have these elliptical areas.

Average total length, 0.864 mm; length of antennae about 0.224 mm. Width of elliptical areas on under side of abdominal segments 0.016 mm, and length, 0.032 mm.

*Color Variation*.—The newly emerged adult differs considerably in coloration from the fully matured individual. This differentiation led Reuter (1891) to establish a variety *abdominalis* of *Heliothrips haemorrhoidalis* which Pomeyrol (1928) pointed out as merely the newly emerged adult of *H. haemorrhoidalis* which had not hardened and taken on its adult coloration. When just emerged, the thorax of *Hercothrips fasciatus* is of a dirty yellowish brown with a darker head. The red markings so characteristic of the larva and pupa can still be seen faintly at the sides of the abdomen. The antennae and legs are pale but have

the dark-brown areas very distinct. The eyes and ocelli are a dark brownish red. The wing bands are clearly demarked. The black pigment appears to be first deposited at the sides of the abdomen, obliterating the red blotches, and spreads gradually over the remainder of the body.

Whenever larvae are forced to feed in the direct sunlight, they become very highly colored.

## ORIGIN AND DISTRIBUTION

Very little work indeed has been done on the geographical distribution of the Thysanoptera as correlated with the life zones, and even less information is available on the geological distribution of the insects of this order. Doubtless the small size and rather delicate structure of the thrips has resulted in the paucity of specimens given us as fossils today.

In regard to the geographical distribution of the Thysanoptera on the basis of Merriam's life zones, Watson (1926) wrote that the Thysanoptera of North America practically ignore Merriam's zones and that the Thysanopteran fauna of Florida is more closely related to that of Massachusetts and Northern Europe than to that of California. The same author also stated that there was no evidence that the distribution of the Thysanoptera corresponds in the least to the formations or societies as set up and defined by ecologists.

As to the original home of the bean thrips, little can be stated in a definite way. Certain authors have suggested that it is indigenous to California, and collections and the observed habits of the insect show that it exists in this state on the native vegetation far from cultivated crops (Moulton, 1907). Watson (1923) reported this thrips on native vegetation in Florida and inferred that *Hercothrips fasciatus* might be indigenous to Florida. Also Bondar (1924) collected it in Bahia, Brazil, and suggested that it was indigenous in that country. There are scattered records in the literature of this species from the west coast of Mexico, Arizona, and Texas; also some unpublished data (kindly furnished by Dr. W. E. Hinds, Louisiana State University, and by Professor Franklin Sherman of Clemson College, South Carolina) extend the known distribution of the bean thrips to Alabama, Louisiana, and South Carolina. Steinweden and Moulton (1930) reported the collection of one female bean thrips from citrus at Foochow, China. Until there is further evidence, it can only be said that *H. fasciatus* is actually indigenous to the warmer climates.



## NATURE OF INJURY

The injury done by the bean thrips is the direct result of the feeding of the larvae and adults upon plant tissue. In the feeding act the mouth cone is applied to the leaf surface and the initial incision made with the mandibles and their stylets which are protruded from the concave side of the labrum. The long maxillary stylets are then brought into play and used to puncture the deeper cell layers. A rooting motion of the head is employed to enlarge the opening and cause the plant juices to flow more rapidly. The labrum, which bears at the tip a round socket through which the stylets pass, is closely appressed over the lesion and the fluids sucked up. Peterson (1915) wrote concerning the sucking action "The muscles along the meson of the elastic membrane (of the pharynx) contract and dilate the lumen of the pharynx so that a partial vacuum is formed, and into this cavity is sucked the juice in which the tip of the mouth cone is immersed. On the relaxation of the dilating muscles, the elastic membrane forces the food dorsad through the open valve into the oesophagus."

Some authors have stated that the secretions of the salivary glands are toxic to the plant, but this has recently been discredited.

Wardle, Simpson, and MacGill (1927) stated that, in the case of the onion thrips, "The stylets can be protruded beyond the labial rim  $11\mu$  in the case of the mandible and  $27\mu$  for the stylets of the maxillae. Whether the hypopharynx can be protruded is uncertain."

As to the relative amount of injury done by the adults as compared with the larvae, there are no actual figures to present. However, from careful observations, both in the field and the laboratory, on the basis of the area eaten over and the amount of excrement deposited, it appears that the larvae do a great deal more feeding than do the adults. This is as would be expected as the larvae are present in much larger numbers; they are less active and confine their feeding to a limited area; they feed gregariously; and they have to store up sufficient food to carry them through the pupal stage with its demand for stored energy. The adult male does much less feeding than the female.

The actual damage resulting from oviposition in the leaves is very slight. When the egg is deposited, a very small lesion is made by the insertion of the sawlike ovipositor, which is not more than 0.167 mm long and 0.059 mm wide at the base. The scar left in the leaf tissue after the larva has hatched is approximately 0.25 mm long.

The actual damage of the bean thrips is caused, almost entirely, in the case of the pear tree particularly, by the premature and excessive defoliation. This not only weakens the tree for the ensuing season but exposes both the new growth and the fruit to "sun-scalding" in the hottest part of the summer.

Wardle, Simpson, and MacGill (1927) have done some excellent work on the actual nature of injury of the onion thrips to the cotton plant. These authors stated that the lower side of the leaves is preferred on account of the difference in the epidermal thickness. The very old and very young leaves are free from thrips since the older leaves are too tough and the young leaves are put forth ahead of the infestation. They wrote further: "leaf injury consists essentially of necrosis of a patch of mesophyll cells lying immediately below a gash in an epidermal cell." The lesions are divided by these authors into four progressive stages: (1) Palisade layer intact, epidermis intact, and a slight disorganization of the outermost mesophyll layer and air spaces. (2) Mesophyll and lower epidermis become more disorganized. (3) Mesophyll nearly all gone and the palisade layer beginning to shrivel. (4) All tissues, including the upper epidermis, disorganized. This exact succession of stages of the lesions will doubtless vary under different conditions and in different plants.

The injury, as evidenced by the dropping of the pear leaves is first noticed, by the growers in the Berryessa Valley (Napa County) particularly, about the middle of July. The browning and curling of the leaves which are shed is apparently the result of the desiccating effect of the hot sun upon the leaves injured by thrips feeding. The older leaves—that is, the ones earliest infested, seem to drop first. The evidence that uninjured leaves in the direct sun, and badly injured leaves that are well shaded, do not turn brown and drop, seems to substantiate the foregoing conclusion.

As a result of the dying and dropping of large numbers of leaves and the toughness of the older remaining leaves, the thrips are driven to the pear fruit. The author has observed many terminals, entirely naked of leaves, with a single pear hanging from it infested with upwards of 100 thrips larvae. The injury to the fruit is in the nature of ugly scars and minute oily drops of excrement which lower the grade and marketability to a serious degree. Such injury occurs only in very heavily infested local areas where early defoliation is present. Of a total of 5,265 pear fruits counted August 6, 1930, on the Eccleston ranch, Berryessa Valley, at the height of the infestation, 781 showed damage, giving about 15 per cent injury.

## HOST PLANTS

A true host plant is taken to be one on which the egg, larval, and adult stages are found (the pupal stage being passed in the soil). It is impossible to ascertain whether the hosts recorded from various sources are, in this respect, true hosts or not, or whether the adults were there transitorily, or hibernating. In general, the bean thrips is not found on plants that are heavily pubescent; tender succulent plants seem to be preferred as is evidenced by the infestations on the young growth.

It is very doubtful if the bean thrips can survive on pines. Adults were confined on *Pinus radiata* at Davis, and none lived more than three days. The adults reported as taken on pine were probably hibernating.

A large list of host plants is given by Russell (1912*b*), Essig (1915, 1926), and Watson (1923), and the plants listed by them and others are included here with new and additional hosts:

## CROP PLANTS

Alfalfa	Cotton	Peas (garden and
Almond	Grape	cowpeas)
Apple	Kale	Persimmon
Avocado	Lettuce	Potato
Beans	Olive	Prune
Beets	Onions	Radishes
Cabbage	Orange	Swiss chard
Cauliflower	Peach	Tangerine
Clover, red	Pear	Tomatoes
Corn (young shoots)		Turnips

## WILD AND ORNAMENTAL PLANTS

<i>Amaranthus retroflexus</i> (rough pigweed)	<i>Convolvulus arvensis</i> (morning-glory)
<i>Anthemis cotula</i> (mayweed)	<i>Crepis</i> sp. (hawksbeard)
<i>Arundinaria japonica</i> (bamboo)	<i>Echinocystis</i> sp.
<i>Asclepias mexicana</i> (milkweed)	<i>Erigeron canadensis</i> (horseweed)
<i>Aster</i> sp.	<i>Erodium cicutarium</i> (red-stem filaree)
<i>Atriplex</i> sp. (saltbush)	<i>Eschscholtzia californica</i> (California poppy)
<i>Bidens pilosa</i> (bur marigold)	<i>Foeniculum</i> sp. (fennel)
<i>Brassica campestris</i> (common yellow mustard)	<i>Geranium</i> sp. (cranesbill)
<i>Canna</i> sp.	<i>Gnaphalium decurrens</i> var. <i>californicum</i> (California everlasting)
<i>Cassia</i> sp.	<i>Helianthus annuus</i> (common sunflower)
<i>Chenopodium murale</i> (nettle-leaf goosefoot)	<i>Heliotropium curassavicum</i> (Chinese pusley)
<i>Cirsium edule</i> (Indian thistle)	<i>Hemizonia</i> sp. (tarweed)

<i>Iris germanica</i>	<i>Monita perfoliata</i> (miner's lettuce)
<i>Lactuca scariola</i> (prickly lettuce)	<i>Nicotiana glauca</i> (tree tobacco)
<i>Lactuca scariola</i> var. <i>integrata</i>	<i>Polygonum aviculare</i> (wire grass)
<i>Lotus americanus</i> (Spanish clover)	<i>Pueraria hirsuta</i> (Kudzu)
<i>Lotus scoparius</i> (deerweed)	<i>Pyracantha</i> sp. (firethorn)
<i>Lupinus</i> sp.	<i>Sonchus oleraceus</i> (common sow-thistle)
<i>Malva parviflora</i> (mallow)	<i>Stellaria media</i> (common chickweed)
<i>Medicago hispida</i> (bur clover)	<i>Tacsonia mollissima</i> (passion flower)
<i>Melilotus alba</i> (white melilot)	<i>Tropaeolum majus</i> (nasturtium)
<i>Mentha</i> sp. (mint)	<i>Tulipa</i> sp.
<i>Mentzelia laevicaulis</i> (blazing star)	<i>Verbascum virgatum</i> (mullein)
<i>Mirabilis laevis</i> (wishbone bush)	<i>Vicia</i> sp. (wild vetch)

## LIFE HISTORY

*Rearing Methods and Technique.*—Both larvae and adults were collected by sucking them into a miniature wash-bottle apparatus (Bailey, 1932). Plain cellophane (No. 300 permeable) envelopes were used in caging the thrips on host plants. These envelopes were placed over single leaves or entire terminals, cotton was wrapped around the petiole

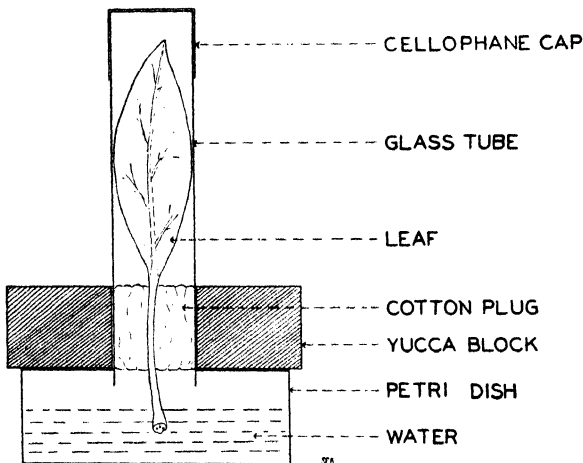


Fig. 4. Type of cage used in rearing thrips in the laboratory.

or twig, and the cage constricted and tied tightly at this point with string. In addition to using potted plants with cellophane cages in the laboratory, a series of glass tubes  $\frac{1}{2}$  to  $\frac{3}{4}$  inch in diameter and 3 inches long were employed as rearing cages (fig. 4). Since the grade of cellophane used is permeable to atmospheric conditions (Bailey, 1931), considerably less condensation of moisture resulted from transpiration and the danger of the thrips' drowning was reduced. This method facilitated

observations, the changing of food, and keeping a supply of water present without allowing the thrips to escape. A camel's-hair brush was used in transferring the small larvae.

The most satisfactory food was found to be pear and bean leaves. Sprigs of alfalfa, while being very satisfactory from many standpoints, prevented accurate counts and observations since the leaves and blossoms were too dense and overlapping.

A specially constructed air-conditioning cabinet was employed in studying the effect of temperature and humidity upon various stages in the life history. Cold chambers, in which the humidity was uncontrolled, were used in obtaining constant temperatures below 50° F.

Further details on the technique used in these studies are given with the experiments which are reported in following pages.

*Hibernation.*—The bean thrips hibernates in the adult stage. Local conditions regulate the place of hibernation as well as the duration of the dormant period. Hibernation seems to be chiefly a temperature reaction since inactivity on the part of the adults can be brought about under controlled conditions by lowering the temperature, and normal activity is resumed upon subsequently raising the temperature. Both the entrance into and the emergence from hibernation is gradual and, even on exceptionally warm days (maximum temperature 75°–80° F) in the winter, they exhibit some activity.

Only a very slight amount of feeding seems to be done during hibernation and practically no excrement is to be seen on the leaves of the plants furnishing the protective quarters. All activity is at a minimum, and they are found nearly always clustered together and will, if disturbed, move about very slowly. The abdomen of the hibernating adults appears shrunken and flat and the wings in many cases seem to be stuck together. When dormant adults were taken into the laboratory and warmed up, the males seemed to react first, the females being much more sluggish. When sufficiently warmed up, they began to hop about and commence feeding. Temperatures as low as 16° F appeared to have little effect on the hibernating adults in the field.

About the last of October or the first of November, the adults become inactive and seek out hibernating quarters, migrating chiefly to plants that are still green. Egg laying, copulation, and finally feeding cease and the adults begin to cluster in protected places. The overwintering population appears to suffer a heavy mortality, and many individuals have been found dead in drops of water standing on the leaves, and large numbers of dead have been observed stuck to wet leaf surfaces. Only those adults extremely well protected seem to survive the drench-



ing rains of the winter season. By the last of March the survivors become active and gradually migrate to alfalfa and various weeds, such as prickly lettuce, sow-thistle, and filaree, occurring nearby.

This insect then passes about five months of the year in hibernation, i. e., from the latter part of October or the first of November to the last of March or the first of April.

At Davis in the fall of 1929, adult bean thrips were found hibernating at the base and in the angles of onion stalks in an isolated seedbed, and on the under side along the midrib of Swiss chard leaves, particularly those close to the ground. In January, February, and March, 1930, adults were found hibernating at the base of iris leaves, and here again on those leaves close to the ground.

Mr. F. H. Wymore collected hibernating adult bean thrips on orange leaves infested with soft brown scale (*Coccus hesperidum* L.) at Davis, December 16, 1929. The thrips were huddled among the scales and some were even in the emergence holes of the parasitized scales.

In Monticello (Napa County) in October, November, and December, 1929, adults were found on red-stem filaree in the pear orchards. During the winter it is very difficult to find the remaining scattered adults on account of the profuse growth of weeds and their wet, matted condition.

In the winter of 1930-31 a more extensive search for hibernating adult bean thrips resulted in finding large numbers hibernating on various ornamentals at Davis. In addition to iris, adults were collected from the under side of the leaves of roses, *Pyracantha*, *Viburnum*, and *Canna*.

L. A. Whitney (1930) reported that, during inspection work in Hawaii in December, 1929, hibernating adults of *Hercothrips fasciatus* were found on shipments of persimmons, tangerines, and oranges from California. Russell (1912*b*) reported that Professor Lawrence Bruner took hibernating adult bean thrips from the navel end of oranges at Lincoln, Nebraska, February 14, 1899, and at Urbana, Illinois, March, 1907. In both instances the oranges were from California.

*Activities of the Newly Emerged Adult.*—It has been noticed in the laboratory, from a very large number of pupae collected in the field as mature larvae, that newly emerged adults are inactive for about 24 hours. When a number of mature larvae pupate on the same day, the females, in every case observed, emerge as adults 10-30 hours before the males. Mating has never been observed on the part of newly emerged adults without first having fed. They remain quiet for the first day, probably for the hardening up of the exoskeleton and full deposition of

pigment. An occasional individual will live as long as six days after emerging without food or water.

Feeding begins in earnest on the second day, and is increased on the third day. Copulation has been observed on the second day in a few instances and oviposition has been found to occur as early as the third day after emergence. These activities seem to vary, however, with the temperature. Oviposition has been observed in a number of cases on the fourth day after emerging on the part of unfertilized females.

*Ability of the Adult to Burrow Up Out of the Soil.*—The adult bean thrips do not have much ability to force their way up through the soil. It was thought that the newly emerged adults crawl to the surface through cracks and openings in the soil rather than actually forcing their way through the compact soil. If the structure and position of the soil particles are altered after the mature larvae have found a suitable place in which to pupate, it was shown that the adults cannot make their way to the surface, and consequently die.

Mature larvae and pupae were placed in pint jars and coarse or fine sand from  $\frac{1}{2}$  to 3 inches in depth carefully spread over them. Over the mouth of each jar was stretched white cotton cloth held tightly in place by a rubber band. The jars were set on a laboratory table and inspected daily for newly emerged adults by examining the under side of the cloths, care being taken not to disturb the soil. Jars Nos. 1 and 2 contained pupae that had darkened up and were about ready to emerge. Jars 3, 4, and 5 contained prepupae and pupae, and jars 6, 7, and 8, mature larvae only. A summary of the experiments is given in the accompanying tabulation.

Jar	Number of forms	Kind and depth of covering
1	10	$\frac{1}{2}$ inch fine soil
2	12	$\frac{1}{2}$ inch coarse soil
3	10	1 inch fine soil
4	20	1 inch coarse soil
5	10	3 inches coarse soil
6	35	1 inch fine soil
7	12	1 inch coarse soil
8	32	3 inches coarse soil

Normally the adults should have appeared in one day from jars 1 and 2 and in about six days from jars 6, 7, and 8. However, after ten days no adults had appeared and we were forced to assume that they could not burrow to the surface when covered in this manner.

in *Hercothrips fasciatus*. In the laboratory under controlled conditions, in the greenhouse, and in the field the bean thrips has been found to reproduce bisexually and, when no males are present, the unfertilized females produce only male progeny.

In these experiments the females were obtained by collecting mature larvae in the field or by raising them in the laboratory, allowing them to pupate, and isolating the female pupae as soon as they had developed sufficiently to enable the sexes to be distinguished. These virgin females upon emerging were placed on uninfected leaves of beans or pear. Their progeny were raised to maturity and the sex determined. Cellophane envelopes were used in the field and glass tubes were employed in the laboratory (see section "Rearing Methods and Technique"). The females were changed to fresh leaves every five or eight days with the collecting apparatus or a camel's-hair brush. Individuals classified as "mated" were observed to copulate in vials before being isolated. The mature larvae were allowed to pupate on the cotton plugs both in the field cages and in the glass tubes in the laboratory. Check cages of mated individuals were run in each experiment when the potential parthenogenesis of virgin females was being tested.

During the summers of 1930 and 1931 a total of 22 experiments were conducted with unmated females to determine whether or not they were able to reproduce parthenogenetically. In these experiments a total of 274 unfertilized females were used, all of which gave positive results and the progeny which were reared to maturity from 140 individuals were all males. In the check cages in the field where males were continually present and promiscuous mating took place, the proportion of sexes was 35 per cent males and 65 per cent females, which is only a slightly higher percentage of males than found in the field collections of adults.

A number of females reared in the laboratory were mated once only in vials and then isolated on pear leaves to determine the proportion of sexes in the offspring. The experiment was continued in each case until the death of the female. The larvae hatching out in ten-day intervals were isolated and the sex determined when maturity was reached.

Table 1 gives a summary of this experiment.

TABLE 1  
PROGENY OF FEMALES MATED ONCE SHOWING  
PROPORTION OF SEXES

Female No.	Sex of progeny in successive 10-day periods	
	Females	Males
1 (Length of life, 52 days).....	{ 3	1
	{ 7	0
	{ 6	3
	{ 0	3
	{ 0	7
2 (Length of life, 22 days).....	{ 12	0
	{ 0	12
3 (Length of life, 20 days).....	{ 6	0
	{ 0	5

Females which are mated only once apparently either use up the sperm in fertilizing the eggs laid first or the remaining sperm becomes impotent and the eggs laid later are unfertilized.

Another set of experiments was conducted illustrating the converse of the above. Newly emerged unmated females were isolated on pear leaves for a given number of days; the larvae hatching out were carefully reared, and the sex of the adults determined. The original unmated females were then mated and again isolated and the sex of the offspring determined. A summary of this set of experiments is given in table 2.

TABLE 2  
PROPORTION OF SEXES IN PROGENY OF FEMALES,  
BEFORE AND AFTER MATING

Number of females		Sex of progeny	
		Females	Males
5 {	Unmated, 8-day period.....	0	16
	Mated, 8-day period.....	18	4
5 {	Unmated, 8-day period.....	0	13
	Mated, 8-day period.....	15	4
1 {	Unmated, 27-day period.....	0	16
	Mated, 8-day period.....	3	0
1 {	Unmated, 20-day period.....	0	23
	Mated, 10-day period.....	2	2

The unmated females produced only males and when mated produced nearly all females, the males appearing after mating probably came from eggs which in some manner escaped fertilization.

So, it can be seen that the bean thrips, while normally reproducing bisexually, is capable also of reproducing parthenogenetically. The males are then necessary to the continuation of the species and there is no evidence that a transition is taking place from a bisexual mode to one of parthenogenesis. Doubtless, in nature, females often reproduce parthenogenetically but the progeny in such an event, being all males would insure fertilization of any females remaining and the subsequent production of more females.

*Copulation.*—Copulation in *Hercothrips fasciatus* takes place as has been observed and described in other species by many workers in the suborder Terebrantia. The male being smaller and much more active than the female, when conditions are such as to stimulate copulation, avidly seeks out the female. The male either crawls or hops on to the back of the female and, as Russell (1912*b*) writes, "It then exerts the copulatory organs from the tip of the abdomen and shifts around toward the side of the female, at the same time bending the abdomen under the ventral side of that of the female. The copulating organs are then moved back and forth until they encounter those of the female." The length of time of connection varies from 2 to 10 minutes. At high temperatures (95°–115° F) the complete act is greatly shortened.

*Egg Laying.*—Both temperature and light seem to play important rôles in stimulating or retarding egg laying. Oviposition by the bean thrips is most frequently observed in the early morning and late afternoon. The process of egg laying has been observed to take place on both the upper and lower sides of pear, prune, and prickly-lettuce leaves. The duration of the act seems to vary greatly, depending on the condition of the leaf tissue, being from 4 to 9 minutes.

The act of oviposition begins, after the female has found a suitable place, by arching up the abdomen and extruding the ovipositor at right angles to the body. With a sawing motion the ovipositor is driven its entire length into the leaf and worked forwards and backwards to enlarge the slit. The female then rests before beginning the actual laying of the egg. Contractions and undulatory movements of the abdomen are visible and a firmer grasp of the leaf is taken by the feet. The female appears to labor heavily at the time that the egg passes out of the body and a slight exudation of excrement is sometimes visible. Often, after the ovipositor is withdrawn, a drop of excrement is placed over the slit made in the leaf. Feeding begins soon after the act is completed.

The ovipositor is often caught or wedged in the leaf tissue and the female dies struggling to withdraw it. This seems to be more common on the older leaves. Many attempts at oviposition are made which are unsuccessful, owing, no doubt, to the toughness of the epidermis.

*Proportion of Sexes.*—The proportion of sexes throughout the year does not vary greatly. During the past two years collections and counts have been made to determine the exact relation in numbers between the sexes and the seasonal variation in the proportion if there be any. Collections were made in the field on various hosts throughout the year and counts were also kept of the sex ratio of adults reared in the laboratory. In all, 2,601 adults were counted, of which 1,769 were females and 832 males, which gives a ratio of 68.1 per cent females and 31.9 per cent males.

The proportion of sexes of 485 adults reared in the laboratory was found to be 72.5 per cent females and 27.5 per cent males. The proportion of sexes in greenhouses on beans was found to be 74.4 per cent females and 25.6 per cent males.

Collections of adults were made at Davis throughout the year and sex ratios recorded to determine whether or not there was any seasonal variation in the proportion of sexes in the bean thrips as is known to occur in many other insects. Table 3 gives a summary of the monthly collections and determinations.

TABLE 3  
SEASONAL VARIATION IN PROPORTION OF SEXES

Date	Host	Females	Males	Total	Sex ratio, per cent	
					Females	Males
July 19, 1930	Beans.....	116	40	156	74.3	25.6
Aug. 4, 1930	Prickly lettuce.....	70	33	103	67.9	32.0
Sept. 20, 1930	Canna, iris, and prickly lettuce..	107	64	171	52.5	37.4
Oct. 18, 1930	Canna, iris, and ornamentals.....	97	42	139	69.7	30.2
*Nov. 27, 1930	Canna.....	274	159	433	63.2	36.7
*Dec. 26, 1930	Canna and <i>Viburnum</i> .....	149	77	226	65.9	34.0
*Jan. 24, 1931	Canna and <i>Viburnum</i> .....	58	51	109	53.2	46.7
*Feb. 15, 1931	<i>Viburnum</i> and iris.....	40	29	69	57.1	42.8
Mar. 21, 1931	Prickly lettuce and sow thistle..	60	24	84	71.4	28.5
April 19, 1931	Prickly lettuce.....	50	16	66	75.7	24.2
May 20, 1931	Prickly lettuce.....	159	76	235	67.7	32.2
June 22, 1931	Prickly lettuce.....	80	28	108	74.0	28.9
	Totals.....	1,260	639	1,899	.....	.....
	Average sex ratio.....	.....	.....	.....	66.3	33.6

\* Hibernating period.

It will be seen that the sex ratio does not vary greatly in the greenhouse or in the laboratory from the ratio found to exist in the field under normal conditions. The seasonal variation, although not being great, demand some explanation and the following interpretation is suggested. Russell (1912*b*) thought that since the males were more active than the females they were not so often collected. However, the sex ratio determined from adults reared from confined larvae in the laboratory does not differ greatly from the average sex ratio of all the field collections and very closely approximates field collections made in the same month. It is difficult to explain why the collections made in January and February show a higher percentage of males since the males are normally shorter lived than the females and are apparently more easily killed by low temperatures. A high percentage of males is apparently not required in this thrips, as is the case in some insects, since the males are very active sexually and mate promiscuously. The normal sex ratio of females to males then is about 2 to 1.

*Power of Flight.*—The power of flight is not so strong in the bean thrips as in many of the Terebrantia and consequently it does not migrate so readily as does the pear thrips or wheat thrips. The adult of *fasciatus* has, however, the power of hopping, and this capacity aids greatly in the dispersal of these insects. The wings of adults have been clipped off by the writer and the distance of the hops was never greater than 10 or 12 inches. Before taking off, the end of the abdomen is curled upwards which aids in spreading the wings, and then the insect springs nearly straight up into the air. Without the aid of the wind, the flight is zig-zag or spiral in manner and is usually not more than 3 or 4 feet in distance.

The manner of dispersal in the field is irregular, depending on the amount and direction of the air currents and the source of the original infestation.

*Longevity of the Adult.*—The length of life of the adult bean thrips varies with the season of the year. Hibernating adults live as long as five months and during the hottest part of the summer the average life of the adults in the field is about three weeks. Without food or water the average individual cannot live much longer than 24 hours and, with only water available, an occasional adult will live as long as six days. Newly emerged adults have been observed to exist as long as three days without food.

No actual counts were taken on the numbers of overwintering adults that survived, but during the winter of 1930–31 at Davis it is known

definitely that hibernating adults lived at least five months. The length of the hibernating period will of course vary from year to year and no doubt certain overwintering individuals survive even longer.

The average length of life of 312 adults confined in cellophane cages on pears and beans was 22.1 days.

Also, mature larvae were collected in the field and allowed to pupate on the cotton plugs in shell vials in the laboratory. The adults upon emerging were retained in the vials either without water or with water made available by standing the vial upright in a dish of water, the cotton plug acting as a wick. This raised the humidity in the vial and the adults could obtain water by crawling down upon the saturated cotton. Food was made available to the thrips by the method described under "Rearing Methods and Technique." Of 89 newly emerged adults, the average length of life without food or water was 21.6 hours; and of 277 newly emerged adults, which were given water only, the average length of life was 4.6 days. The average length of life of the adult bean thrips was found to be 15.7 days, when given both food and water, in the laboratory under artificial conditions, which is somewhat shorter than in the field.

*Hatching, and Activities of the Larva.*—Bean thrips larvae have been observed to hatch from the egg on many occasions both in the daytime and at night. Previous to emergence, the egg seems to swell and give the appearance of a minute bump on the leaf. With magnification, the reddish-brown eye spots are often discernible through the egg shell. The egg shell splits lengthwise in the region of the thorax of the mature embryo. The back of the head and thorax of the emerging larva become visible first. The head is then pushed out of the shell and, by undulatory movements, the whole body is raised at right angles to the leaf surface. The emerging larva bends its body over sufficiently to grasp the leaf surface with its feet and pulls the tip of the abdomen entirely out of the egg shell. Feeding commences immediately.

Since the morphology of the mouth parts has not been studied in detail by the writer, it can only be said that, to all appearances, the mouth parts of the larva are similar to those of the adult. As was stated under the section "Nature of Injury" the larva does more feeding than the adult and is also inclined to feed in groups over a small area of leaf or fruit surface, thus leaving the silvery scars which are so characteristic of thrips' damage.

*Distribution on the Host.*—The majority of the larvae are found on the under side of the leaves of the hosts. Possibly they frequent the



under side because the adult female finds the tissue here more favorable for oviposition or the larvae wish to avoid the direct rays of the sun. However, with the general observation in mind, counts were made to determine if such was actually the case. Of a total of 6,463 larvae counted on prickly-lettuce and pear leaves, 3,978, or 61.5 per cent, were found on the under side and the remainder on the upper side of the leaves.

In the case of heavily infested plants or terminals the larvae after having eaten over the entire under surface of the leaf will crawl onto the upper side and there complete their development. Thus, if counts were made from plants heavily infested with thrips early in the season, the majority of larvae would be found on the upper surface. However, under the usual conditions, the largest numbers of larvae are found on the under side of the leaves. In general, it might also be said that as the infestation works upwards on the host through the season, the majority of the large mature larvae are found on the lower leaves and the younger larvae and eggs on the upper ones.

The proportion of larvae to adults found on the host plants presents a condition which goes to show the great mortality present in the larval and pupal stages of this species of thrips. The subject of mortality will be found treated in another section and it is the purpose here to present the data obtained from field counts and observations on the proportion only.

Infestations of bean thrips were examined on pear foliage, beans, alfalfa, Swiss chard, sugar beets, and prickly lettuce, and counts were recorded of the numbers of larvae and adults present. Counts were made of the total number of thrips on individual plants and on individual leaves taken at random. Needless to say, during the fall, winter, and early spring such figures do not apply since there are no larvae between November and April. Over a period of three seasons, on various hosts and in three localities, the percentage of adults varied only from 14.4 per cent to 32.2 per cent. The proportion of larvae to adults on the plant is, then, about 5 to 1.

*Mortality of Mature Larvae, Prepupae, and Pupae in the Soil.*—In making this detailed study of the life history of the bean thrips it was observed that, as is the case very often in nature, there is a very high mortality under natural conditions, and of the large numbers of individuals which are hatched, only a small number reach maturity and reproduce.

That such is true of the bean thrips is very clearly shown by daily counts and careful records kept over a period of nearly two months

during the summer of 1930. A total of 9,143 mature larvae was obtained from the daily counts taken, and of this number 3,542 adult thrips and 83 parasites (*Thripoctenus russelli* Cwfd.) emerged. These figures show a 60.3 per cent mortality (including 0.9 per cent parasitized) of those forms which were in the soil.

The greatest mortality occurs before the mature larvae transform to the prepupal stage. The prepupal stage contributes to the death rate, but to a lesser degree than the larval stage. In the pupal stage there is a slightly higher death rate than in the prepupal stage since the latter is very much shorter. Of a total of 624 mature larvae collected from host plants in the field and reared in the laboratory, 213, or 34.1 per cent, died before molting to the prepupal stage; 58, or 9.3 per cent, died in the prepupal stage; and 103, or 16.5 per cent, died while in the pupal stage. From these figures we obtain a 59.9 per cent mortality, which is very close to that obtained from the field counts.

Some further attempts were made to determine the mortality of those stages which undergo a period in the soil. A wooden box, 12 x 8 inches and 8 inches deep, was filled with coarse soil and buried in the ground beneath a stand of prickly lettuce. The top of the box was made level with the surface of the ground and a wooden cover fitted tightly over it. A hole was bored in the cover through which a known number of mature larvae were introduced. A test tube was then inserted in this hole in the cover. The adults upon emerging are positively phototropic and crawl up into the tube towards the light and thus could be collected and counted. From 300 mature larvae employed, only 114 adults were obtained, thus giving a mortality of 62 per cent of the stages in the soil.

It is rather remarkable that the percentage of mortality in the field in 1930, in the laboratory over a period of several months, and again in the field in 1931, are all so close, varying only from 59.9 to 62 per cent. It appears then that only about 40 per cent of the individuals becoming mature as larvae ever reach the adult stage and the counts illustrating the proportion of larvae to adults (which was found to be 5 to 1) point toward an even higher mortality.

Needless to say, before the larvae reach maturity and drop from the host, various predators, in addition to other factors, take their toll.

## SEASONAL HISTORY

The seasonal history of the bean thrips is not so narrowly defined nor so regular in its cycle as are certain other species of thrips, the pear thrips, for example (Cameron and Treherne, 1918). The generations are not clearly demarked and the seasonal activities as a whole are subject to considerable fluctuation depending upon the climatic factors and their degree of yearly variation. An account of the activities through the seasons can then, only in a general way, follow the insect through its yearly cycle. In addition to there being a yearly variation in the appearance and abundance of the bean thrips, its seasonal history varies to some extent in the different localities of its range, but we are here concerned with the conditions obtaining in central California.

The winter is passed in the adult stage on various host plants, preferably those remaining green throughout the colder months. This period of hibernation (November through March) is not one of complete inactivity. The adults are found singly or in groups on the under side of the leaves of various ornamentals or on filaree, lupine, poppy, miner's lettuce, and other native plants brought up by the first fall rains. The driving rains kill many of the overwintering individuals, unless well protected, by beating them off the hosts and trapping and drowning them among the lodged and sodden plants. During March, prickly lettuce, which is the favorite host, begins to appear and with the first warm days the survivors gradually migrate to this host and other early succulent plants such as sow-thistle and oftentimes alfalfa.

With the rising daily mean temperature (55°–60° F) of spring the adults begin to copulate and lay the eggs for the first generation. The adults continue to feed and oviposit for several weeks, but it is doubtful if many of them survive long enough to be included with the adults of the first generation and contribute to the second.

The length of the various stages in the life history and the number of generations were determined from cages placed on the host plants in the field.

The eggs, which are deposited in the leaf tissue require a long period of incubation, probably as much as 20 days, in the early spring. By the last of April larvae can be found in small numbers on prickly lettuce, filaree, California poppy, sow-thistle, etc., but the adults have become very scarce. The larvae mature in about three weeks and drop to the ground, crawl down cracks and openings in the soil to a depth of several inches and there transform. The prepupal and pupal stages together

require 10 days or more. By the latter part of May the adults begin to appear in numbers to complete the first generation.

The newly emerged adults crawl up to the surface by way of the cracks and openings in the soil and find their way back onto the same wild plants and there lay the eggs for the second generation. The larvae of the second generation can be found about the first of June. The life of the adults during April and May is about four weeks and they continue to feed and oviposit over this entire period.



Fig. 5. Prickly lettuce between bean field and orchard providing favorable conditions for midsummer infestation of bean thrips.

The second generation completes its cycle in about a month, whereas the first generation requires about six weeks. From the first of June on, there is such an overlapping of generations that there are no marked broods.

During June, July, and August, a generation is completed about every three weeks. At the high summer temperatures (daily mean temperature of about 72° F) the length of incubation of the egg is about 7 days, the first and second larval stages together extend over about 10 days, and the pupal forms pass about 5 days in the soil.

Relatively few bean thrips are found on any plants in the summer other than prickly lettuce; i. e., where cultivated crops or ornamentals are not near by. About the first of July, this plant begins to throw out its inflorescence and the plant loses its succulence (fig. 5). The adults emerging at this time are forced to seek new hosts and there are to be found very few wild plants suitable. However, the variety *integrata* of

*Lactuca scariola* germinates much later than the species and there is thus a scattering second crop of prickly lettuce. In addition, blazing star, *Mentzelia laevicaulis*, and a few other plants also serve as hosts during the late summer. The adults congregate upon these plants which are capable of supporting them through the late summer and early fall, provided, of course, crops are not at hand.

In September and early October there is another, the sixth, and possibly a partial seventh, generation. Shortly after the first of October the larvae disappear rapidly with the approaching cool, damp weather.

With one generation in April and May, a second and perhaps a partial third in June, one and one-half generations in both July and August, and one generation in September and early October, there are, normally, six or perhaps seven generations a year in central California.

The fall rains bring up a new crop of native plants, such as filaree, lupine, etc., which the remaining adults seek out and upon which they pass the winter.

There are two critical periods in the seasonal history of this thrips and they are both dependent on the amount of precipitation, which of course directly influences the abundance and condition of the native hosts. If the fall rains are either light or late in arriving, the adults have no succulent host capable of supporting them previous to hibernation and consequently suffer a high mortality. Again in the spring, in years of low rainfall, if there is no rain after March and the weather warms up rapidly, the wild plants are usually small and mature early. This results in a serious condition for the bean thrips, for even though a hot, dry climate is most favorable to the species, if the hosts are small and mature early, a high mortality prevails.

It is during dry years or in localities that present a semiarid condition normally that the bean thrips becomes of economic importance. When the native hosts begin to dry in early summer, the newly emerging adults at this time seek any other hosts available as beets, alfalfa, beans, pears, cotton, etc. The initial infestation builds up rapidly as the high mean temperature and low humidity make for ideal conditions for multiplication and by late summer severe damage results to such crops, particularly in nonirrigated sections.

A detailed study of the seasonal history of the bean thrips in pear orchards has been carried on during the past three seasons, and a brief account appropriately finds a place here.

The major portion of the work was done in the Berryessa Valley (Napa County), but additional information was obtained from pear-

growing sections in Sacramento, Yolo, and Lake counties (Lewis, 1928, 1929, and Smith, 1930).

In the winter months the hibernating adult thrips are very difficult to find in the pear orchard environment. The floor of the orchard is at this time usually covered with a dense growth of weeds composing a covercrop. The few surviving adults are usually found on filaree and additional individuals often may be obtained from other weeds by general sweeping with a net. Almost invariably, wherever a prickly-lettuce plant is to be found, in thrips-infested localities, the adults are found congregated thereon in the late winter. About the last of March the covercrop of weeds is plowed under, and the adults which have successfully survived the winter become concentrated on any prickly-lettuce plants not destroyed by plowing in the orchards, and most frequently on those stands which so commonly spring up along the roadsides. The first and probably the second generations of the season are passed on these weeds.

From this point on, up to the time of the initial infestation of the pear trees, the activities of the thrips are difficult to follow accurately but an attempt is made here to analyze the succession of events at this time as brought out by varying local conditions.

In view of the facts that the early infestations are very local in the orchards and that the bean thrips has no strong powers of flight, migration from adjacent fields can perhaps be discounted. Also, there is no direct evidence that a prevailing wind is a factor of any great import in the distribution of this species of thrips.

It has consistently been apparent that the early infestations on the pear trees can be definitely correlated with the presence of wild hosts in very local areas. Those trees with limbs close to or actually touching the ground or those with sucker growth are the first to be infested. With the varying degree to which orchardists employ cultural practices, comparisons have been afforded in this study between orchards extremely well kept and those which have been somewhat neglected. From these comparisons it has been apparent that those orchards which were the most untidy in respect to weed growth, and those not well pruned, experienced the worst damage from bean thrips.

This matter of the presence or absence of weeds offers perhaps two possible explanations for the ultimate damage to the orchards. One explanation suggests that the early cultivation and removal of the prickly lettuce and other weeds forces the few adults present to migrate to the pear trees and, with an initial infestation of small numbers, some weeks are necessary for a sufficient population to build up to cause

severe damage. On the other hand, if many wild hosts are present in and about the orchard, a large population of thrips builds up thereon (since the native hosts are preferred to the pear) and when these plants are killed, or mature, a forced migration to the pears takes place. Such an infestation takes place later in the season than in the case cited above, but the initial infestation in the second case is much greater and the damage, while showing up somewhat later, is more severe.

Whatever may be the exact succession of events, the thrips are usually found on the pear trees during the latter part of June. Throughout the following weeks the infestation which starts on the lowest branches gradually works upward and outward through the trees and by the last of the summer thrips are found, in severe cases, 15–20 feet high on the outermost terminals. In general, it is about the middle of July that the orchardists first notice the presence of the bean thrips on account of the dropping of dead leaves.

The third, fourth, and fifth generations and probably a partial sixth, depending on the season, are passed on the pear. By the first of October the leaves begin to fall and from then on the larvae disappear rapidly. The adults begin to seek out the remaining wild hosts as *Lactuca scariola*, *Lupinus*, and the first fall plants, chiefly filaree, on which to feed occasionally and subsequently pass the winter.

## PARASITES AND PREDATORS

In spite of the abundance of the bean thrips and the habit of the larvae of feeding gregariously and unprotected on the leaf surface, the number or abundance of natural enemies is not great. We find representatives of six orders of insects, mites, and a nematode among the parasites and predators of *Hercothrips fasciatus*. Of the internal parasites there have been discovered to date only one hymenopteran, *Thripoctenus russelli* Cwfd., and a nematode; these are both parasitic on the immature forms only. No secondary parasites have as yet been reported.

The larvae, being rather sluggish in their movements and unprotected by a heavy exoskeleton as are the adults, become the easy prey of their enemies. The adults, however, are not entirely immune from the attack of predators for, when the larvae are scarce, both the nymphs and adults of *Orius insidiosus* var. *tricolor* White and the larvae of *Aeolothrips fasciatus* (Linn.) will prey upon the adult bean thrips. This was observed more commonly in the laboratory than in the field, since the larvae are seldom reduced sufficiently in numbers, with the exception

of the spring and fall, to induce a condition where the predators are forced to feed on the adults.

In reviewing the literature on the Thysanoptera, we find listed as natural enemies: ladybird beetles—species of *Megilla*, *Scymnus*, and *Hippodamia*; of syrphid larvae—*Syrphus* and *Sphaerophoria* sp.; *Chrysopa* spp.; *Orius* (*Triphleps*) spp., *Anthocoris* sp.; several predaceous thrips—*Aeolothrips* and *Scolothrips* sp. As internal parasites, there have been listed *Thripoctenus* spp., and *Tetrastichus* spp. In addition to the above-mentioned natural enemies, nematodes, fungi, mites, and spiders are of minor importance.

In central California during three seasons, the writer has made careful observations in the field and some studies in the laboratory to determine the relation between the bean thrips and certain insects commonly found associated with it. Only one internal parasite, *Thripoctenus russelli* Cwfd., has been found; the remainder are predators chiefly upon the immature forms and are the larva of *Chrysopa californica* Coq., *Orius* (*Triphleps*) *insidiosus* var. *tristicolor* White, both adult and nymph, the larva of *Hippodamia convergens* Guerin, the larva of *Aeolothrips kuwanai* Moulton (predacious on eggs and larvae), and the larva of *Aeolothrips fasciatus* (Linn.).

Adults of *Orius tristicolor* were placed with bean thrips which were confined in vials on prickly-lettuce leaves and observed over a period of weeks. Both the nymphs and adults attacked the smaller thrips larvae first, then the larger ones and finally the adults if no larvae remained. This predator is very active and may be seen to run up to a thrips and stick its beak into the head, thorax, or abdomen with no apparent aim. The struggling victim is often held down by the fore feet of the anthocorid, gradually the body of the larva may be seen to shrink and after 5 to 20 minutes, according to the size of the larva, the predator withdraws its rostrum and sets about cleaning its entire body. If disturbed while feeding, *Orius* will frequently run about or even fly with a thrips larva impaled on its needlelike beak. In the laboratory the adult predators consumed about one larva an hour and the nymphs appeared even more voracious. Sakimura (1930) reported that *Orius tristicolor* was predacious on the alfalfa thrips *Frankliniella occidentalis* (Perg.) to the extent of 26 to 33 per cent at Greenwood, Utah.

This anthocorid appears to prefer the larva of the common flower thrips to the bean thrips. However, since the flower thrips becomes greatly reduced in numbers during the hot summer weather, *Orius* is forced to feed upon the bean thrips in many localities. This predator winters over as an adult in rubbish and early in the spring they may be



found feeding upon the larvae of the flower thrips. As the season progresses both the adults and nymphs become more abundant, and the eggs may be found in small yellowish-white clusters on the leaves of the host supporting the thrips. In the middle of the summer the bean thrips serve as food, and then again in the cooler weather of the fall, as the population of flower thrips builds up again and the bean thrips begin to disappear, *Orius* transfers its activities back to *Frankliniella* spp. The adult predators have lived as long as two weeks in confinement and the nymphal stage, during the summer, is about ten days. In certain localities this predator undoubtedly effects a considerable check on the infestation of bean thrips.

The larvae of *Hippodamia convergens* Guerin and *Chrysopa californica* Coq. in the spring may be found preying upon the first *Hercothrips fasciatus* larvae appearing, usually on prickly lettuce, sow-thistle, or alfalfa. These two predators do not seem to seriously affect the thrips populations and are of no great importance as natural enemies.

Of the predacious Thysanoptera two species have been found in the larval stage to prey upon the bean thrips. These two species are *Aeolothrips fasciatus* (Linn.) and *Aeolothrips kuwanai* Moulton. Adults of *A. fasciatus* confined in vials with bean thrips in the laboratory and in cellophane cages on the host plants in the field have never been seen to prey upon the bean thrips in any stage. The large yellow larva of *A. fasciatus*, however, are very voracious and inflict considerable damage in colonies of bean thrips larvae in the spring. Under confined conditions both larvae and adults of *Hercothrips fasciatus* are preyed upon. After the hot weather comes on, *A. fasciatus* can hardly be found. The larvae take two to three weeks to mature and, in the protection of curled leaves or debris near the host plants, they pupate. Very little is known further of the habits of this species. While it is common, no large numbers ever seem to be present and it is therefore not an important factor in reducing the bean thrips.

Likewise, the adult of *Aeolothrips kuwanai* has never been observed to prey upon the bean thrips either in the field or under artificial conditions. The long, slender, maroon-colored larvae, however, feed extensively on both eggs and larvae of *Hercothrips fasciatus*. On pears, prunes, and prickly lettuce, these predacious thrips larvae have been seen to walk about seeking the bean thrips eggs in the leaf tissue, and, upon finding one, to sink its mouth cone into the slight swelling raised on the leaf surface by the egg and suck up its contents. These larvae occasionally found their way into life-history cages and no bean thrips larvae hatched from such infested cages. In addition to feeding upon

the eggs, they prey on the larvae but will not attack the adults. The pupae of *Aeolothrips kuwanai* transform on the host plant in curled leaves, abandoned spider webs, etc. While this species also is rather common, it never reaches the abundance necessary to be an important predator and is seldom seen at the height of summer when the bean thrips infestations are most severe.

The most important natural enemy of the bean thrips is probably the internal parasite *Thripoctenus russelli*. This parasite, a very minute chalcidoid of the subfamily *Tetrastichinae*, was first discovered by H. M. Russell at Compton, California, in 1910 (Russell 1911, 1912a), and was described the following year by J. C. Crawford.

This parasite has not been studied in detail by the writer, but some interesting facts have been noted which add to what is already known of it. The adult parasites have been found during every month in the year in the open in association with the bean thrips. During the winter the adults have been found among the hibernating adults thrips on the under side of the leaves of various hosts. In the spring they are to be seen actively running about among the first generation of thrips larvae on prickly lettuce, filaree, sow-thistle, etc. During the summer they appear to be even more scarce than in the spring, but in September and October with the falling off of the thrips population there seems to be a sudden increase in the parasites.

This parasite has been observed to oviposit in the larger larvae only, and the act of oviposition required from 15 seconds to 4 minutes. On several occasions a parasite was seen to feed at the wound after ovipositing. Eggs are deposited both in the thorax and abdomen of the thrips larva but usually at the side of the abdomen. Attempts to oviposit are many times unsuccessful owing to the violent struggling of the larva. Parasitized larvae usually molt to the prepupal stage but never reach the pupal stage. The length of the egg plus the larval stage of the parasite averaged about 7.5 days while the pupal period averaged about 14 days during June and July, 1931. Adult parasites kept alive as long as 5 days at 30° F constant temperature were capable of ovipositing upon removal.

Russell (1912b) wrote that the parasitism of *Hercothrips fasciatus* in southern California ranged as high as 70 per cent. In central California the writer would estimate that there was, during the seasons of 1929-1931, very little over 5 per cent parasitism by *Thripoctenus russelli*; in fact, during June, July, and August, 1930, upwards of 10,000 thrips larvae were collected and counted and only 0.9 per cent parasitism was present. However, as was stated above, with the falling-off of the thrips

population and the apparent increase of parasites, counts taken in the fall would have shown a much higher percentage.

It might be well to call attention to the fact that Bagnall (1913) reported *T. russelli* in England parasitic on *Taeniothrips primulae*, *Physothrips atratus*, *Physothrips ericae*, *Oxythrips parviceps*, *Thrips tabaci*, *Thrips palustris*, and *Frankliniella intonsa*. Cotterell (1927) wrote of a new chalcid parasite of the cacao thrips, *Heliothrips rubro-cincta* (Giard), and his report of the habits of the parasite leads us to believe that it is *Thripoctenus russelli* or a very close relative. In addition there is a report by Waterson (1923) of an internal chalcid parasite, *Tetrastichus thripophonus*, of a thrips (no species given) in Trinidad, British West Indies.

Four species only of the suborder *Tubulifera* have been reported as parasitized. Williams (1916) described a new species of chalcid parasite, *Thripoctenus nubilipennis*, taken from *Megalothrips spinosus* and *Cryptothrips rectangularis* at Forest Hills, Mass. Mason (1922) reported a new parasite, an undescribed species of the genus *Tetrastichus*, from *Cryptothrips laurcli* on bay trees in Florida. None of these parasites, even though so closely related, have as yet been reported from *Hercothrips fasciatus* or other members of its genus. Barnes (1930) described a new gall midge, *Thripsobremia liothrips* Barnes (Cecidomyiidae), which is predacious on *Liothrips urichi* Karny in Trinidad. He wrote "This midge, besides being interesting because of its structural character, is noteworthy as being the second gall midge recorded as feeding on thrips. The other species is *Adelgimyza thripidiperda* Del Guercio, which is predacious on *Phloeothrips oleae* Costa, in Italy. This species was described (1918 and 1919) from the female and is only provisionally placed in the genus *Adelgimyza*."

Concerning the remaining natural enemies of the bean thrips—nematodes, fungi, mites and spiders—little can be said definitely. Russell (1912*b*) wrote that Mr. P. R. Jones had found a "nematode parasite working in the full-grown larvae of the bean thrips" at Lindsay, California. Fungi have often been observed attacking thrips in all stages, particularly under conditions of high humidity. However, the writer has never been able to determine whether the fungi attack the thrips while they are normally active or only after they have become weakened or have died. The latter is more probable.

Only two species of mites, *Hypoaspis* (*Laelaps*) *macropilis* Bks. and *Anystis agilis* Bks. have been reported from North America as preying on thrips.

Adult bean thrips are very frequently found dead in spiders' webs on the host plants but no detailed study has been made of this relation and its proportional importance among the natural factors in reducing this thrips.

## EFFECTS OF TEMPERATURE AND MOISTURE ON THE LIFE STAGES

*Range of Activities of the Adult as Limited by Temperature.*—The temperature range of various activities of the adult bean thrips was determined by placing the insects in an air-conditioning cabinet and varying the temperature at a constant rate of 1° F in three minutes. The humidity was held at  $64 \pm 4$  per cent. The adults were collected from prickly lettuce and confined in glass tubes with cellophane caps over the upper end and prickly-lettuce leaves were used as food. The activities of the thrips were under continuous observation through the glass door of the cabinet. In one series of experiments the temperature was lowered until all adults became dormant and then raised again; in another series, the temperature was raised until the lethal point was reached. From 30 to 100 adults were used in each experiment. The "optimum" temperatures were considered to be those at which the greatest number of individuals exhibited activity. No data were obtained on the temperature range in which oviposition takes place since the bean thrips does not oviposit readily under artificial conditions. No relative differences in the activities of the sexes was noticed. (The males are at all times more active than the females.) The following tabulation gives a summary of the temperature range of various activities as determined under the above conditions.

	Temperature range of activity	Optimum temperature
Feeding .....	58°F -114°F	77°F -90°F
Copulation .....	62° -116°	80° -97°
Hopping .....	70° -118°	No marked optimum
Crawling .....	47° -120°	No marked optimum
Staggering .....	117° - death	.....
Dormancy .....	..... - 45°	.....
Death .....	118° -122.5°	.....

The above data indicate that the activities of the adult occur over a wide range of temperature. There appears to be a tendency on the part of the adults to cluster when the temperature approaches that of dor-

mancy but this activity was not considered marked enough to include in the data. The optimum temperature range of all activity is between 75° and 90° F. The age of the thrips was not considered in making these determinations, and in determining the lethal point the factor of time of exposure was not weighed.

Considerable variation among individuals was observed in the range of activities, but the figures presented are an index to the range of temperature in which feeding, sex activity, etc., take place. The limitations of activity as listed are not to be taken as inflexible since these experiments were conducted under artificial conditions. In the field, with its complex of environmental factors, marked variances would no doubt be found.

*Effect of Temperature on Emergence of the Adult from the Soil.*—During the period from June 20, 1930, to August 9, 1930, daily emergence records were kept on the number of adults emerging from beneath each of four heavily infested prickly-lettuce plants which were kept under observation throughout the season. The plants were situated about 30 feet from the edge of a pear orchard. Closely woven white cloths, two feet square, were fitted tightly around the base of the plants and were weighted down along the edges with fine sand. At 7:00 A.M. and 5:00 P.M. each day the cloths were carefully rolled back and the newly emerged adults found clinging to the under side of the cloths were counted. (The adults were unable to penetrate the cloth or burrow out under the edges.) The mature larvae which had dropped from the plants onto the upper side of the cloths were first counted, and later, after the newly emerged adults had been counted, were placed on the soil and the cloths replaced. In this manner, over an area of 4 square feet beneath each plant, the daily emergence of adults was determined.

The infestation of thrips on these plants reached its maximum about July 11–15, and by the first of August the plants were flowering profusely and the population dropped off rapidly as a result of the loss of succulence.

A total of 3,542 adults was obtained from June 20 to August 9, 1930. The greater percentage of the adults emerged between the hours of 5:00 P.M. and 7:00 A.M. Of the total of 3,542 adults counted, 2,705, or 76.4 per cent, emerged between 5:00 P.M. and 7:00 A.M. There is no marked difference in the sexes in this reaction since, of a total of 2,505 females, 1,868, or 74.6 per cent, emerged between 5:00 P.M. and 7:00 A.M.; and of a total of 1,037 males, 837, or 80.7 per cent, emerged between 5:00 P.M. and 7:00 A.M. Not only do the totals of the daily counts during this period show this to be the case, but the individual daily records also.

The temperature of the soil at the surface apparently is an important factor and has a marked influence upon the emergence of the adults. During July and August, soil surface temperatures of  $120^{\circ}$ – $140^{\circ}$  F during the day on unshaded areas are not uncommon and we have shown that such high temperatures are fatal to the adults; also, we have shown that no pupae survive within three inches of the soil surface if unshaded. It has been observed that when adults do emerge during the warmer parts of the day, they either succumb or immediately hop or fly onto the plants nearby and there find a temperature which is  $20^{\circ}$ – $30^{\circ}$  lower. On the other hand, the adults which emerge during the night, early morning, or evening do not so quickly seek the plants and remain resting quietly on the soil, crawl about slowly, and by a series of hops and flights reach the nearby plants. When such newly emerged adults are placed in the sun, they immediately become highly active and attempt to escape. It must be realized that often where the host plant is low or spreading, and thus shading the ground, the lethal soil surface temperatures are seldom reached. Furthermore, the counts and observations show that the majority of the adults emerge when the soil surface temperatures are such that they are enabled to safely gain the hosts.

The daily emergence records of adults throughout the period from June 20 to August 9, 1930, were plotted against the daily mean temperature and the curves smoothed by taking a five-day sliding average. There is, however, not nearly so close a correlation with the fluctuations in the mean temperature as was found in the case of the mature larvae dropping to the soil.

*Rate of Egg-Laying as Influenced by Temperature.*—The influence of temperature on egg-laying proved to be a very difficult point upon which to obtain any accurate data. The leaves of various hosts, as beans, pear, lettuce, beets, etc., were used, but none gave as good results as alfalfa, and it was found to be extremely difficult to make accurate counts of eggs in such a type of plant. Trouble was also had in keeping the cut leaves fresh enough to induce oviposition.

The information gathered on the effect of temperature on oviposition and the rate of egg-laying seems to be somewhat contradictory. In the field, oviposition has continually been observed most frequently in the morning between 7:00 A.M. and 9:00 A.M. and in the late afternoon. On the other hand, in the laboratory under controlled conditions the greatest rate of oviposition was found to obtain at  $100^{\circ}$  F, at which temperature, however, all activity is greatly increased. This seemingly contradictory evidence would indicate that other factors are of more relative importance than temperature.

In the experiment conducted to determine the effect of various constant temperatures on the rate of oviposition the humidity was held constant at 40 per cent (with a fluctuation of 4 per cent) in each case. Pear leaves were used as a host plant and were changed as often as necessary, according to the temperature. The leaves were inspected daily under a binocular for eggs. The results are tabulated below:

Number of females	Duration of experiment	Temperature (deg. Fahr.)	Total number of eggs laid
40	20 days	60	0
40	16 days	70	1
40	10 days	80	9
50	10 days	90	1
25	12 days	100	65

*Longevity of the Adult in Relation to Temperature.*—The longevity of the adult bean thrips was determined at various constant temperatures and the results obtained seem to approximate very closely the conditions found in the field.

In this set of experiments an air-conditioning cabinet was used in which the temperature was held constant. The humidity was held at 40 per cent at each temperature. At temperatures above 50° F, pear leaves were used as food; and at temperatures below 50° F, no food was necessary since the thrips were inactive. Cold chambers in which the humidity was uncontrolled were employed in obtaining temperatures below 60° F. The adults held at low temperatures were kept in shell vials and removed and counted twice a day—about five minutes being necessary for the survivors to warm up and become active enough to be separated from the dead. Table 4 lists the results.

TABLE 4  
LONGEVITY OF ADULT BEAN THRIPS AT CONSTANT  
TEMPERATURES

Number of adults		Temperature (deg. Fahr.)	Average length of life	Number of adults living maximum time	
Males	Females			Males	Females
17	64	10	57.0 hours	0	7
101	267	30	104.6 hours	2	17
14	45	32	66.2 hours	0	5
36	69	40	267.0 hours	3	13
20	60	50	67.7 hours	0	5
29	45	62	72.0 hours	0	7
0	10	70	15.0 days	0	2
30	94	100	7.0 days	0	16

For inactive or hibernating individuals there appears to be an optimum survival temperature of about 40° F. Constant temperatures below freezing produce 100 per cent mortality in 2-4 days. However, when held at temperatures above that which produced complete dormancy and below that producing normal activity, without food, the adults survive no more than 3 days. Then again, with food, higher temperatures (i.e., 100° F) shorten their life as is usually the case when metabolism is markedly increased. The maximum length of life of 15 days was obtained at 70° F.

In central California temperatures of 40° F and below are not normally experienced over any period of days and, under natural conditions, the hibernating thrips could no doubt easily survive much lower temperatures for a few hours. There are no data from laboratory experiments to support a comparison of the longevity of adults at constant and fluctuating temperatures. However, since the bean thrips is found in certain sections of Idaho and Utah having much lower mean winter temperatures than central California, it seems reasonable to believe that the adults can withstand much lower temperatures than these constant-temperature experiments indicate. There is no information on the place or manner in which the adults hibernate at high altitudes or in localities where severe winter conditions obtain. When they do survive, it is doubtless in extremely well protected situations and there is probably very high mortality.

*Effect of Temperature on the Larval Stage.*—The atmospheric temperature is one of the most important factors in the development and rate of growth of the bean thrips larva. Several weeks are necessary for the development of the larva in the spring and fall when the mean temperatures are considerably lower than during the summer, at which time the larval stage lasts only 10 days or less.

Newly emerged larvae and larvae that had just completed the first molt were used in these experiments. The larvae were obtained from a supply reared in the laboratory on pear and bean leaves as well as from prickly lettuce in the field. The rearing cage composed of a glass tube, cellophane cap, etc., as described previously, was used. Pear leaves were used as food, and some difficulty was experienced in keeping the leaves fresh at high temperatures. The glass tubes and leaves were inspected daily under a binocular for molted skins. The cabinet alone was employed in these experiments and the humidity was held constant in each case at 40 per cent. The results of these experiments are presented in table 5.



TABLE 5  
EFFECT OF TEMPERATURE ON THE LARVAL STAGE

Number of mature larvae	Temperature, (deg. Fahr.)	Average length of instars, days		Average length of larval stage, days	Per cent total development per day	Average mortality, per cent
		First	Second			
60	60	18.0	15.0	33.0	3.3	57.0
60	70	5.4	8.2	13.6	7.4	31.6
65	80	5.0	4.0	9.0	11.1	21.5
35	90	4.3	3.8	8.1	12.3	52.4
45	100	3.0	2.0	5.0	20.0	81.5

The lowest average mortality of 21.5 per cent occurred at 80° F and would perhaps indicate that this temperature is the most favorable to larval development.

The rate of development of the larva, as was true of the pupal stage, increased directly with an increase in the temperature. The theoretical zero point of development is at about 50° F (fig. 6, *B*). In this graph, which illustrates the per cent of total development per day of the larva, the straight line is fitted by averaging the three lower points and the two upper ones and drawing the line through the points thus determined. The curve in figure 6, *A* was fitted to the length-of-stage curve by merely reading off and plotting the values from the straight line (fig. 6, *B*) ; for example, at 80° F the percentage of total development per day is 10.5 per cent and it would thus take the larva 9.5 + days, theoretically, to complete its development while the experimental figure is 9 days. The rate of development is expressed after the manner used by Parker (1930). The circles represent the experimental data and the crosses the theoretical figures.

With these data, as with the data on the effect of temperature on the pupal stage, in the "reciprocal growth curve" (Sanderson and Peairs, 1913) a straight line was fitted to the per cent curve (fig. 6, *B*) and the corresponding hyperbola fitted to the length-of-stage curve (fig. 6, *A*).

*Effect of Temperature on the Dropping of Mature Larvae from the Host to the Soil.*—It was observed from daily counts taken from July 20 to August 9, 1930, that the majority of the mature bean thrips larvae dropped from the host to the soil between 5 P.M. and 7 A.M. Heavily infested prickly-lettuce plants were the source of the counts, and cloths were spread beneath them to make the collection of the mature larvae possible. Counts were taken daily at 7 A.M. and 5 P.M.

Of the total of 9,143 larvae counted over this period, 7,658, or 87 per cent, dropped to the ground between 5 P.M. and 7 A.M. There was a downward trend in the daily counts as the result of the maturing of the host and the subsequent decrease in the thrips' population.

In order to determine more specifically during which hours the largest number of larvae drop to the ground, counts were taken every 2 hours for a 24-hour period.

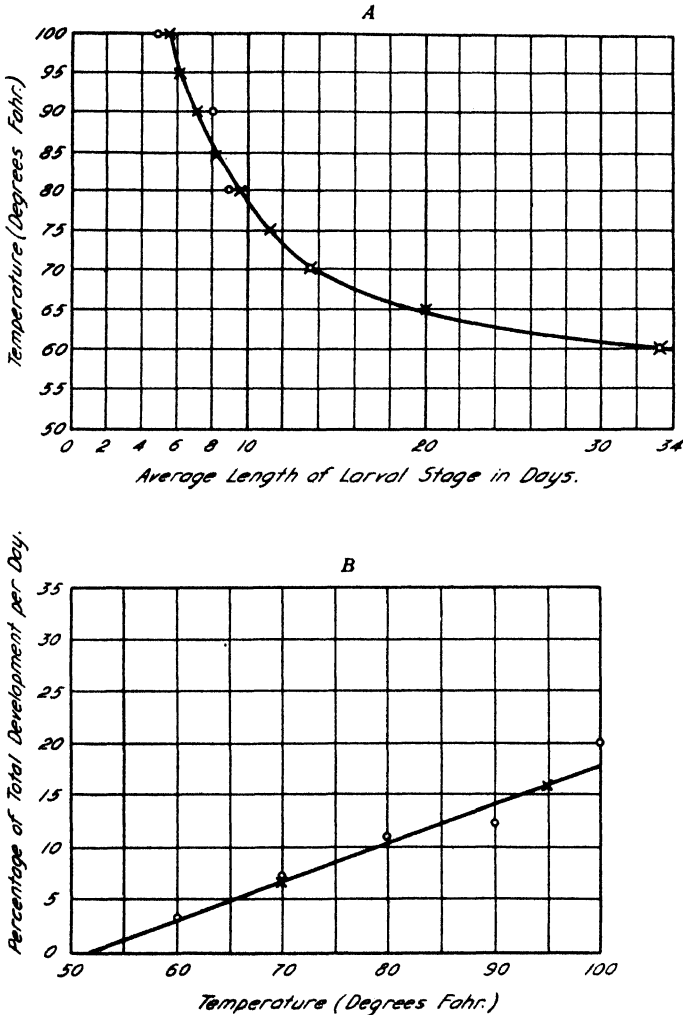


Fig. 6. Growth curves of the bean thrips. *A*, average length of the larval stage at various constant temperatures. *B*, per cent total development per day at various constant temperatures. The circles represent the experimental data and the crosses the theoretical figures.

When the counts are represented graphically (fig. 7), it is more clearly seen that the largest number of mature larvae dropped to the soil between 6 P.M. and 10 P.M. and that a second and much smaller peak was reached between 6 A.M. and 10 A.M. From this, it appears that there was either an optimum atmospheric temperature between 65° and 75° F, at which the greatest number of mature thrips dropped from the host or a diurnal rhythm in respect to this activity. The soil-surface tempera-

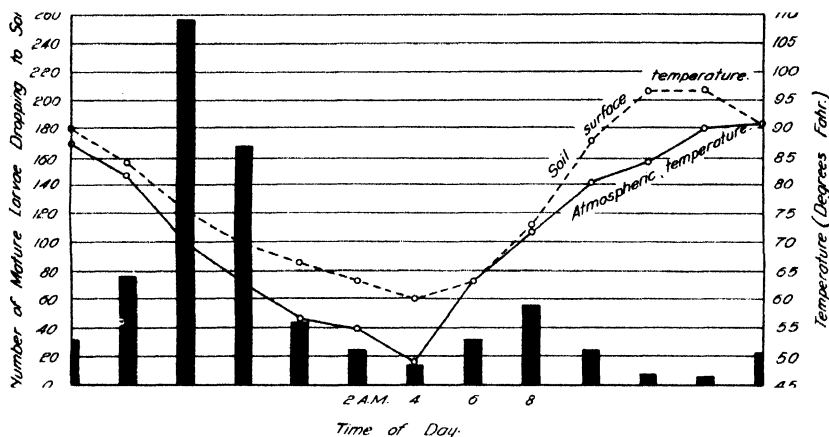


Fig. 7. Number of mature larvae dropping to the soil over a 24-hour period with atmospheric and soil-surface temperatures for period.

tures are given to show that at the time the majority of the larvae drop, temperatures lethal to them are not present at the surface of the ground and that sufficient time elapses for them to find a suitable place for pupation before the heat becomes too great.

*Effect of Temperature on the Pupal Stage.*—Temperature plays a most important part in influencing pupation, both as to the length of the stage and the rate of development, as well as the mortality occurring during this period.

The constant temperatures from 60° F to 110° F were obtained with an air-conditioning cabinet in which the humidity was held constant at  $40 \pm 4$  per cent throughout the experiments. The constant temperatures below 60° F were obtained by the use of cold chambers in which the humidity could not be regulated. The mature larvae were collected in the field from the top of cloths spread beneath prickly-lettuce plants. The larvae were placed in vials and a cap of cellophane (No. 300 permeable) was stretched over the open end and held in place by an elastic band. The vials were examined daily and every few hours at the higher temperatures, under a binocular. The data obtained are given in table 6.

TABLE 6  
EFFECT OF TEMPERATURE ON THE PUPAL STAGE

Number of mature larvae	Temperature (deg. Fahr.)	Average length of prepupal period, days	Average length of pupal period, days	Average length of prepupal and pupal periods, days	Per cent total development per day	Average mortality, per cent
50	28	.....	.....	.....	0 0	100 0
316	30	.....	.....	.....	0 0	100 0
50	40	.....	.....	.....	0 0	100 0
60	50	12 0	.....	.....	0 0	100 0
75	60	9.5	14 0	23.5	4.2	94.0
80	70	1.6	9.3	10.9	9.1	78.6
100	80	1.0	3 0	4 0	25.0	50 0
75	90	0.9	2 4	3 3	30.3	50 0
108	100	1.0	1.8	2.9	34.4	65.7
50	110	0.8	.....	.....	0 0	100 0

The rate of development is expressed after the manner used by Parker (1930), that is, as the percentage of a total development of the stage completed in one day of 24 hours. For example, if 5 days are required to complete the pupal stage at 85° F the rate of development at 85° is 20 per cent of the total development per day. The length of the stage plotted against the temperature gives a typical growth curve (fig. 8, *A*). When the percentage of total development per day is plotted against the temperature (fig. 8, *B*), the same type of curve is obtained as that termed a "reciprocal growth curve" by Sanderson and Peairs (1913).

It should be kept in mind that the bean thrips pupates in the soil and that soil temperatures are much more constant than atmospheric temperatures.

From the results it is apparent that the rate of development of the pupal stage increased directly with the temperature, from 60° to 100° F. Above and below this range 100 per cent mortality resulted while the lowest mortality prevailed equally at 80° and 90° F.

*Depth of Pupation in the Soil and the Effect of Soil Temperatures on Pupation.*—A careful inspection of soil beneath pear trees heavily infested with bean thrips revealed pupae, prepupae, and mature larvae at a depth of 3 to 6 inches. They were found in niches and cracks of the clumps of dirt, singly, and sometimes clustered together. The questions were then raised: how deep into the soil do the larvae make their way and is there an optimum depth which they seek?

Experiments were carried on both in the field and in the laboratory in an attempt to answer these questions. In all the experiments glass tubes with an inside diameter of  $\frac{3}{16}$  inch were used. Tubes of lengths from 4

to 15 inches were employed and individual tubes were filled entirely with fine or coarse soil particles or with a piece of roughly twisted string. The purpose of using the string was to serve as a support for the larvae in crawling downward, to insure an unobstructed path, and also provide suitable places in which to pupate. In all cases the tubes were placed vertically and cotton plugs were used at both ends. Mature larvae were collected in the field and introduced at the upper end of the tube. When the larvae were thus confined, the depth to which they crawled could be

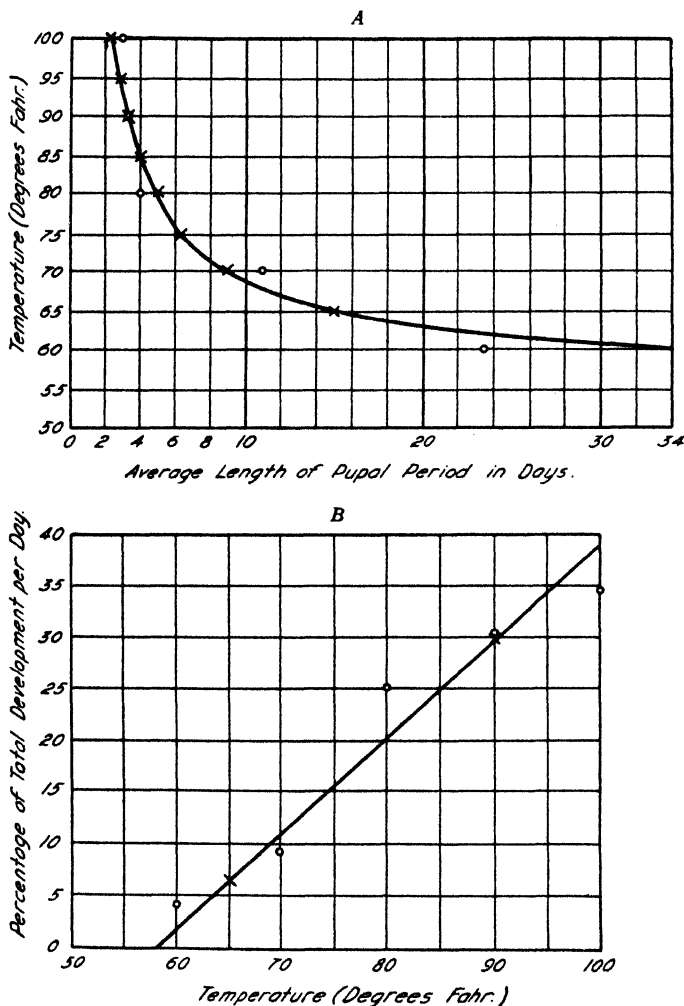


Fig. 8. Growth curves of the bean thrips. *A*, average length of the pupal stage at various constant temperatures. *B*, per cent total development per day at various constant temperatures. The circles represent the experimental data and the crosses the theoretical figures.

measured, the number of larvae pupating at each depth counted, and the number of adults emerging recorded.

Fifty mature larvae were introduced into each of two tubes 6 inches long, one filled with fine, closely packed sand and the other with coarse, irregularly distributed particles of soil. These tubes were placed upright on a laboratory table and then covered to shut out the light. There was a high mortality among the group of larvae pupating in the fine sand. Five days later only 11 adults emerged from the tube containing sand. From the other tube which contained coarse soil, five days after the mature larvae had chosen their place of pupation, 31 adults emerged. This experiment was repeated several times and the results were the same in every case; i. e., the larvae could not penetrate the fine sand and there was a very high mortality; and, also, the larvae will crawl down among the soil particles until they reach an obstruction, whether it be  $\frac{1}{2}$ , 1, 2, 3 inches, or more, at which point they will pupate.

Twenty prepupae were placed in a tube containing coarse soil and then observed. The prepupae, not being so active as the larvae, did not attempt to crawl down to the extent that the larvae did and came to rest in the first agreeable place that offered itself. No prepupae reached a depth greater than  $\frac{1}{2}$  inch.

Two tubes, one 10 and the other 15 inches in length, with a piece of loosely twisted string running the entire length, were also employed. Fifty larvae were introduced into each tube. The majority of the larvae in each tube found their way to the bottom and there pupated in small groups in folds of the string. A few individuals were distributed the entire length of the tubes. The indication from these observations is that the mature larvae will crawl down until they find a place to their liking in which to pupate, irrespective of the depth, unless they meet with some obstruction.

In the orchard  $\frac{3}{16}$ -inch glass tubes of 5, 10, and 15 inches in length were used. These tubes were buried vertically with the upper end of the tube level with the soil surface. Either coarse soil or string was used within the individual tubes. As in the laboratory the larvae were found to make their way down to 15 inches depth or until their path became obstructed. Likewise, larvae were also found distributed the entire length of the tubes. Fifty mature larvae were used in each case and the experiment was repeated several times during July and August, 1930.

The outstanding observation was made that in no case did larvae attempting, or forced, to pupate within about 3 inches of the soil surface during these months, survive.

A. Smith (1929) has shown that soil surface temperatures of 120° F are common during the summer months at Davis, California, and that at 3 inches below the surface temperatures of 90°–95° are fairly constant during the day. Thus it is not strange that bean thrips pupae seldom survive above in a depth of 3 inches in unshaded areas. Below this depth the mean daily soil temperature is considerably lower and subject to much less fluctuation which would provide a very suitable condition for pupation. It must be kept in mind, however, that where the soil is shaded by low or spreading plants that such lethal soil temperatures do not prevail and that pupation is possible very near to or even on the surface of the ground. This actually does occur, for pupae have been found in debris on the soil surface in dense stands of prickly lettuce.

*Effect of Humidity on the Bean Thrips.*—As is often the case in experimental work, it proved to be much more difficult to obtain data upon the effect of humidity than upon the effect of temperature on the insect. Also, normal activity seems to occur over a wide range of humidity, and it is only the condition of extreme dryness or of very high humidity that produces any marked effect on the various activities of the bean thrips. A high humidity is apparently unfavorable to this insect. Also it is in the summer when the rainfall is practically nil and in those localities where a minimum atmospheric humidity obtains that this thrips works its greatest damage.

*Range of Activities of the Adult as Limited by Humidity.*—In determining the effect of humidity on the activities of the adult the air-conditioning cabinet was again employed and the insects were confined in vials on prickly-lettuce leaves in the same manner as was described above. No fewer than 100 thrips were used in each set of experiments. The temperature was held constant at 89° F, which temperature favors all activity, and the wet-bulb temperature raised from 60° F to 88° F (giving an increase of relative humidity from 13.5 to 97.5 per cent) at the rate of 1° rise of the wet-bulb temperature every 5 minutes. The "optimum" humidity, as was the case with optimum temperature, was considered to be the range in which the largest number of individuals were active. No oviposition took place during this series of experiments and no differences of note were observed in the activities of the females as compared with the males. In the tabulation below will be found the approximate limitations of the various activities.

It is apparent that humidity does not play such an important part in the activities of the adult thrips as does temperature. The humidity range within which the various activities were observed to occur is ex-

tremely wide. The optimum atmospheric humidity for this insect appears to be somewhat less than 40 per cent, but in no case are the limits of activity so clearly marked as in the case of temperatures. This statement is based upon observations made in the field in addition to laboratory experiments.

	Range, per cent relative humidity	Optimum range, per cent
Feeding .....	31.0-82.0	31.0-68.0
Copulation .....	13.5-97.5	13.5-31.0
Hopping .....	13.5-93.0	13.5-31.0
Crawling .....	13.5-93.0	Uniform through- out range

No deaths occurred on the part of any of the adults used in these experiments at either extreme of humidity. However, when the humidity was below 15 per cent all individuals were highly active and more or less erratic in all their movements. On the other hand, when in a highly saturated atmosphere they became rather sluggish and exhibited no great activity of any nature.

*Effect of Humidity on the Egg, Larva, and Pupa.*—The egg more than any other stage in the life history requires a high and uniform humidity. As was stated above, the egg is deposited in the leaf tissue and in this environment it is constantly surrounded by a high humidity. It has been noted that when the water balance of the host plant is upset and plasmolysis results, the eggs within the leaf tissue fail to hatch. In addition to the loss of water, the shrinking tissues tighten around the egg and doubtless prevent hatching in event the embryo matures. When eggs were deposited on the leaf surface or even upon the wall of a vial, as has happened in the laboratory on several occasions, they slowly shriveled up and never hatched. The chorion is very delicate and when thus exposed desiccation proved fatal.

Experiments conducted under controlled conditions indicated there was apparently no effect on the rate of development of the larva in the range from 22 to 60 per cent relative humidity (the temperature being constant at 90° and 100° F). Such a variation in humidity might have some effect at lower temperatures, but it is very doubtful. The average length of the first larval instar at 100° F was three days both at 22 and at 60 per cent relative humidity, and the average length of the second instar was two days. At a constant temperature of 90° F, and at both 22 and 60 per cent relative humidity, the average lengths of the first and second larval instars were 4.3 and 3.8 days, respectively. In addition



to there being no noticeable change in the rate of development or length of instars there was no apparent effect of a 38 per cent change (22–60 per cent) of humidity upon the feeding or general activity of the larva.

Under normal conditions the atmospheric humidity does not play a direct part in the environment of the pupa and the matter of moisture is governed by the percentage of moisture in the soil. No attempt has been made in these studies to determine the effect of soil moisture upon the pupa but a small amount of data has been obtained upon the effect of various constant humidities.

Constant humidities of 22, 40, and 95 per cent were obtained with an air-conditioning cabinet and the temperature was held constant at 100° F in each experiment. The mature larvae were collected and confined in the manner previously described. Fifty larvae were used in each experiment. The average length of prepupal period was one day and for the pupal period two days at all humidities. The average mortalities at 22, 40, and 90 per cent humidity were 50.0, 65.7, and 61.0 per cent, respectively.

Humidity had very little effect on the length of the pupal stage at 100° F, and the variation in the percentage of mortality at the different humidities is not particularly significant.

Another type of experiment was tried in which  $\frac{3}{16}$ -inch glass tubes 6 inches in length were filled with coarse dirt and plugged with cotton at both ends. Mature larvae were introduced at one end and were allowed to seek out suitable places in which to pupate. The tubes were then placed upright in a shallow dish of water. The water rose slowly, saturating the soil and thoroughly wetting the mature larvae and prepupae. Puddling the soil resulted in trapping many individuals in droplets of water and thoroughly coating them with mud. The majority of those in direct contact with water died in about 24 hours and began to disintegrate rapidly while many others were attacked by a fungus. A total of 120 mature larvae were used and by the end of six days all were dead, only 12 transforming to the prepupal stage.

*Resistance of the Adult to Submergence in Water.*—The adult bean thrips is somewhat resistant to submergence in water and can remain alive for as long as one hour when completely submerged. Some experiments were conducted to determine the resistance of the adults to water. Ten adults were placed in each of six vials which were filled with tap water and inverted. The thrips were observed to crawl around on the bottom and up and down the sides of the vials while completely submerged. Small bubbles of air clung to the hairs of the body and to the

wings. They gradually became weaker and succumbed—two individuals surviving as long as one hour under water. The adult thrips swims readily on the surface by fanning the wings and moving the legs and could easily gain the sides of the vial or petri dish and clamber out unless forcibly submerged in some manner, so in the above experiments a layer of cotton was placed on the surface of the water.

In addition to the above experiments the adult bean thrips were studied in the field during a drenching rain. The beating action of the rain appears to be responsible for the heavy mortality that occurs in the field after a storm. A driving rain washes the adults off the leaves onto the ground. They can also be found dead, adhering to the wet leaf surface and actually in drops of the rain water remaining on the plant. If thoroughly wet, the wings become stuck together and cannot be extended until they are dried. When in or on the ground a thrips adult quickly becomes embedded in the mud or crushed by the shifting and settling of the soil particles.

### SUMMARY

The bean thrips was first collected in Yuba County, California, in November, 1894. Its original home is unknown.

The known distribution records include Mexico, Brazil, China, and the following states in North America: California, Nevada, Idaho, Arizona, Texas, Louisiana, Alabama, Florida, and South Carolina.

The primary injury done by *Hercothrips fasciatus* is the extraction of fluids from the host plants which, in nonirrigated localities and at high temperatures, results in the rapid desiccation of the injured tissues.

The host list includes many native plants and crops. The favorite wild host is the prickly lettuce, *Lactuca scariola*, and the crops most commonly injured are beans, cotton, and pears.

Winter is passed in the adult stage chiefly on the under side of leaves of plants remaining green and offering protection.

The method of reproduction is both bisexual and parthenogenetic; fertilized eggs produce females and unfertilized eggs males. The normal sex ratio of females to males is about 2 to 1.

About the last of March the overwintering adults migrate to prickly lettuce, sow-thistle, etc., and two generations are usually passed on these native hosts in April, May, and early June. During midsummer a generation is completed in about three weeks. There are, then, from April to October, six or seven generations, according to the monthly mean temperature.

With the drying-up of the native vegetation the bean thrips is forced to seek new food plants and thus about the last of June, or in early July, crops become infested.

The eggs are inserted in the plant tissue and appear as minute bumps on the leaf surface. The larva has two stages, molting but once on the host. Upon maturing, the larva drops to the ground and seeks a suitable niche in the soil in which to pupate. The depth of penetration depends on the type and structure of the soil. The mature larva after molting enters a short prepupal stage in which the wing stubs become visible. The prepupa molts and then enters the true pupal stage. In the prepupal and pupal stages the insect is mobile but takes no food. After casting the pupal skin the sexually mature adult, fully winged and pigmented, finds its way to the surface via the openings in the soil. The hosts are gained by short hops and flights.

During the summer months, in central California, the length of the egg stage is about 7 days, the first and second larval stages together are about 10 days, and the pupal forms pass about 5 days in the soil. The preoviposition period is about 3 or 4 days.

The natural mortality of the immature stages is about 60 per cent.

There is only one known internal insect parasite of the bean thrips; namely, *Thripoctenus russelli* Cwfd. The chief predator is *Orius insidiosus* var. *tristicolor* White. Aside from these, the other natural enemies have little affect on the normal thrips population.

The adults are active between about 50° F and 117° F, the optimum range of activity being between 75° F and 90° F.

The rate of development of the larva, as is true of the pupal stage, increases directly with an increase in temperature. The theoretical zero point of development is at about 50° F.

The majority of the larvae drop from the host to the soil at a time when temperatures lethal to them are not present at the surface of the ground.

Pupae seldom survive in the soil in unshaded areas if they have not penetrated at least 3 inches beneath the surface.

The relative humidity range of adult activity is very wide; the optimum appears to be somewhat less than 40 per cent.

Both pupa and adult are comparatively susceptible to drowning.

## ACKNOWLEDGMENTS

To Dr. Stanley B. Freeborn, the writer wishes to express appreciation for fore-sighted guidance and timely counsel in directing the major part of this investigation. The advice of Dr. E. C. Van Dyke and Professor E. O. Essig in directing certain phases of the work has been invaluable. Professor W. B. Herms, Dr. T. I. Storer, and Dr. W. C. Cook have made many suggestions which have aided very materially in the preparation of this paper. The writer is indebted to Mr. L. M. Smith for his cooperation in carrying on some of the field studies.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

JULY, 1933

No. 13

## THE EPIDEMIOLOGY OF FIG SPOILAGE<sup>1</sup>

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### INTRODUCTION

The high percentage of spoilage which occurs in figs grown for drying has been the subject of much investigation. It is generally recognized that this trouble originates internally in the hollow, fleshy body of the fig. (fig. 1) while it is still on the tree in an immature state. In California three specific types of spoilage are distinguished by growers and packers of figs. These are popularly designated as "smut and mold," "souring," and "endosepsis." "Smut" is often considered as a distinct disease. All of these are caused by common saprophytic microorganisms which in some manner are able to invade the central cavity of the fruit previous to its maturity. The possibility of control of spoilage in figs is closely tied up with the question of *how* and *when* these molds, bacteria, and yeasts get into the fig. Of particular importance is the problem of the relation of insects to the transmission and effects of these organisms.

The disease called endosepsis has not been considered in the present work since its etiology and epidemiology were thoroughly established by Caldis (1927), who showed that this particular type of spoilage affects only caprified (pollinated) figs, that it is caused by the fungus *Fusarium moniliforme* Sheld., and that it is transmitted exclusively by the fig-caprifying (pollen-carrying) insect *Blastophaga psenes* L. The types of spoilage regarding the transmission of which there is still uncertainty are the others above-mentioned, smut and mold, and souring. The former trouble is characterized by the presence inside the ripe fig of a mass of moldy material, representing various fungus types like *Alternaria*,

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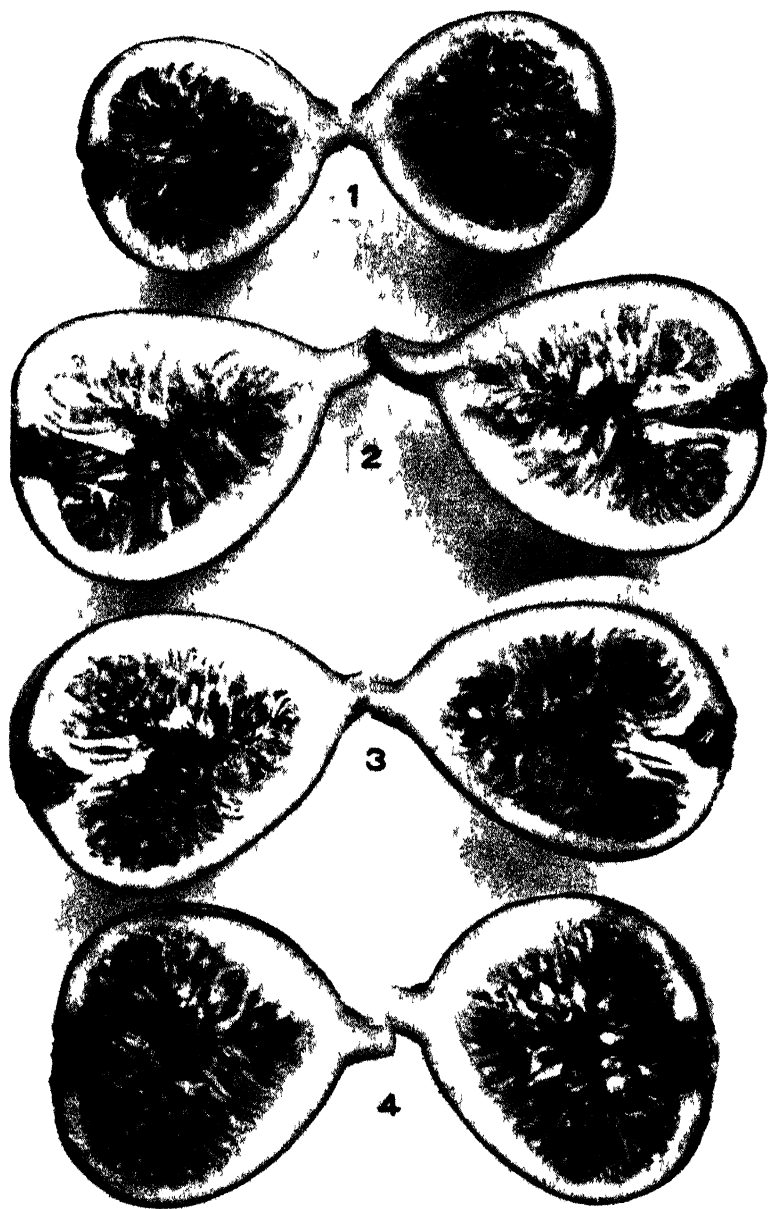


Fig 1 Stages of fig development Nos 1 to 4 (From Bul 387 )

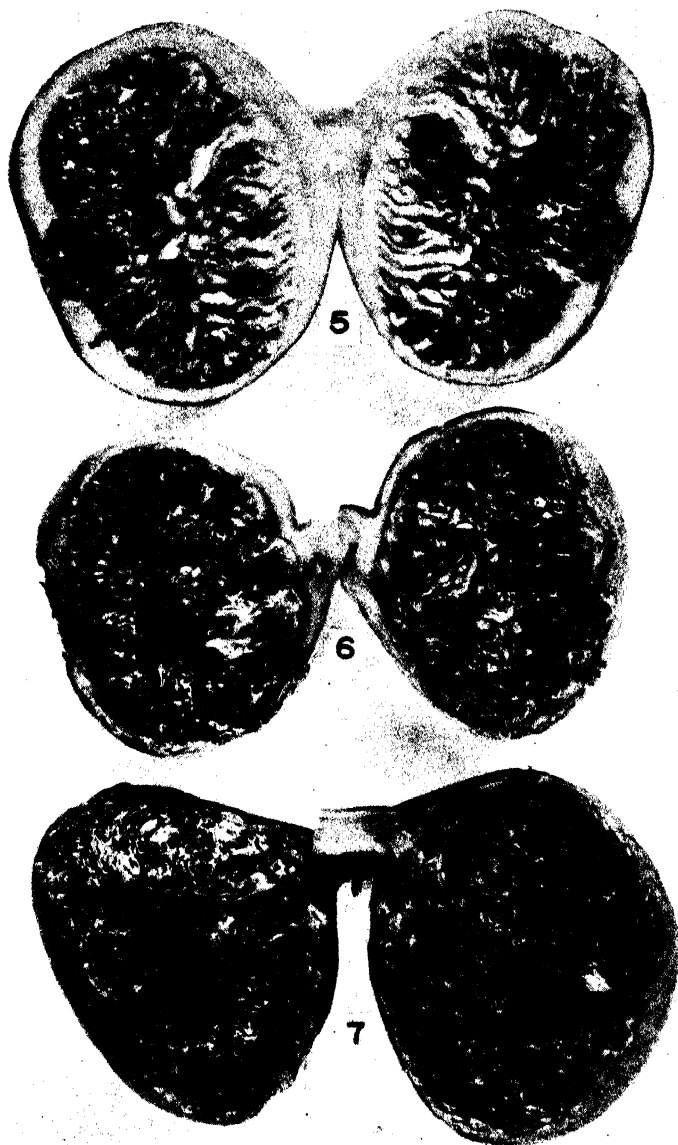


Fig. 2. Stages of fig development Nos. 5 to 7. (From Bul. 387.)

*Aspergillus*, *Cladosporium*, *Hormodendrum*, *Macrosporium*, and *Penicillium*. "Smut" is the name specifically applied to the type of fig spoilage caused by the black fungus *Aspergillus niger* v. Tieg. It is in nowise different from the other types of molding except for its characteristic appearance. Souring is a wet, gassy fermentation of the contents of the fig, supposedly caused by certain yeasts. "Soft rot," a decay of figs on the tree, caused by species of *Rhizopus* or *Mucor* is also a rather typical form of spoilage which is sometimes fairly abundant.

*Previous Work on Fig Spoilage.*—Newton B. Pierce, as early as 1892, suggested the relation of cryptogamic microorganisms and insect carriers to fig souring. Eisen (1901) came to similar conclusions and was the first to call attention to the possible function of the eye of the fig as a barrier to insects and microorganisms entering the interior cavity. Howard (1901) and Condit (1919) also suggested insect transmission of fig souring. Condit (1917) and Hodgson (1918) mentioned a similar possibility in the case of fig smut. Coit (1921), on the other hand, expressed the opinion "Inasmuch as the atmosphere is filled with spores of many kinds of yeasts, molds, smuts and bacteria, and since the eye of the Smyrna fig is open, it is unavoidable that these agents gain access to the interior of the majority of the figs."

In all the work referred to, the idea of insect transmission had to do with scavenger insects, particularly the dried-fruit beetle (*Carpophilus*) and the vinegar fly (*Drosophila*), both of which are very common in ripening figs and decaying fruit. Phillips (Phillips, Smith, and Smith, 1925) undertook the first comprehensive investigation of the subject by means of cultures and systematic laboratory methods. Second-crop Adriatic figs were classed into ten successive stages of maturity, based on the opening of the eye, and the interior of large numbers of figs of each stage was examined. The particular object of this work was the study of the smut disease. The examination of nearly 10,000 figs in this investigation was made, mostly with the hand lens and microscope, for the purpose of detecting the earliest development of *Aspergillus*. Only a comparatively small number of the figs were cultured before the opening of the eye. From this study it was concluded that *A. niger* is not present in figs until after the eye opens (stage 5, fig. 2) and the fig is nearly mature.

When green, immature figs with closed eyes were inoculated with spores of the smut fungus, very active infection and decay resulted. This was found to be true in the Mission and Kadota fig varieties as well as in the Adriatic. Since, under natural conditions, figs in the early stages are not attacked by these mold fungi and since those of the

Mission and Kadota varieties are practically never affected, the conclusion was again drawn that spores are not present in figs before the eyes open. These investigators also found that if the internal tissues of the fig were injured, as with a needle or pipette, in the process of inoculation, infection was more apt to result.

On the basis of all this work Phillips, Smith, and Smith (1925) concluded that "Under summer conditions in the San Joaquin Valley, before the eye of the Adriatic fig opens and the fruit begins to soften, the interior cavity is sterile and neither smut spores nor any other organisms enter." Since a large percentage of the immature figs were not cultured the word "sterile" is apparently used here in a comparative sense to indicate freedom from tissue-destroying fungus colonies visible to the eye or microscope, rather than absolute sterility. In mature figs after the eyes had opened (stage 5, fig. 2) the fruit from some trees showed as high as 50 per cent infection with *Aspergillus niger*. Many figs which were cultured after the eyes opened showed a considerable variety of fungi, *Aspergillus*, *Rhizopus*, *Alternaria*, *Cladosporium*, *Penicillium*, *Hormodendrum*, various species of yeast, and a number of forms of bacteria. From rather circumstantial evidence it was concluded that the usual carrier of spores of *Aspergillus* and other microorganisms into ripening figs after the eye opens, is the dried-fruit beetle (*Carpophilus*). In a previous article (Smith and Phillips, 1922) the statement is made that "Ants, fruit flies and beetles are able to make their way into very green figs with closed eyes, but of course the major part of these visitations occurs after the fruit becomes attractive to them."

Caldis (1927) reported culturing 274 figs of eight parthenocarpic varieties previous to the opening of the eye (stages 1-3, fig. 1), and found them all sterile. Of these figs, 154 were of the Adriatic variety, 67 of these being first crop and 87 of the second crop. By caging *Carpophilus* beetles on ripening figs on the tree, Caldís found that of 54 figs confined in 7 cages with beetles, 50 per cent soured; while of 663 figs in 128 cages with no beetles, none soured. This work was done in two different seasons and in two places.

Hansen (1929) first suggested the importance of thrips as vectors of fig-spoilage organisms and the possibility of their introducing infections before the opening of the eye. In several thousand hard, green figs of four varieties, collected from various parts of California in May, 1928, slightly in excess of 20 per cent were found to be infested with thrips. Figs collected at this time would be of the first crop which ripens in June. Commercial drying figs come entirely from the second crop and commence to form in May and to ripen in August. In 1929, thrips were

again found by Hansen to be common in immature figs. Concerning the cryptogamic flora of the thrips-infested figs collected in May he reports "The interior of 200 of the figs showing evidence of insect invasion were cultured individually on nutrient media to determine their cryptogamic flora. Each of the 200 thrips-infested figs yielded one or more of the following organisms: various species of bacteria, *Rhizopus* spp., *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Verticillium* spp., *Spicaria* sp., *Hormodendrum* spp., and a number of yeasts.<sup>4</sup> The 10 figs showing no evidence of insect invasion yielded no cryptogamic flora in culture." Smith and Hansen (1931) state that "Culturing of thrips taken from figs has repeatedly given the same results, namely, that they carry an abundant flora of yeasts, bacteria, and mold fungi." They also cite several instances of crops of figs which showed a high percentage of smut and mold, correlated with an abundance of thrips in the figs, but no beetles. The thrips yielded in culture the same flora found in the figs.

Smith and Hansen also directed attention to a new vector of fig-spoilage organisms, of the type known as predaceous mites. Several species of these almost microscopic creatures are now known to be common in the interior of green figs where they apparently prey upon the fig mite (*Eriophyes fici* Ewing). Smith and Hansen showed by cultures that the bodies of predaceous mites taken from overwintering caprifigs were contaminated with the same molds and other organisms that are carried by thrips.

Hansen and Davey (1932) studied in more detail the relation of thrips and predaceous mites to cryptogamic infestation of figs. Green, second-crop Adriatic figs were taken at various maturity stages from the hazelnut size up to the time when the eye scales begin to loosen. This was done in four different fig districts at intervals of 4 to 7 days between July 1 and August 15, 1930. All the figs were split open and examined for insect infestation.

In regard to cryptogamic infestation, the following statement is made: "During the progress of this examination mites and thrips taken from the interior of the figs were cultured on nutrient agar from time to time to determine the abundance and diversity of flora carried by them. . . . The cryptogamic flora . . . on mites and thrips cultured included the following species named in the order of the frequency of their occurrence: Miscellaneous fungi, bacteria, *Hormodendrum* spp., *Asper-*

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<sup>4</sup> We are able to state from personal knowledge and information that the "yeasts" referred to in the work of Hansen and associates with thrips and predaceous mites were yeastlike fungi, not those forms which cause fermentation and souring in figs. These yeastlike fungi form a membranous, wrinkled, dry surface growth on solid media.

*gillus* spp., *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp., *Acrostalagmus* sp., and a few yeasts<sup>5</sup>." The figs themselves were not cultured in this work; the exact dates or fig stages at which the mites and thrips were cultured is not stated but the inference is that it was all before the eyes were open.

In the same work Hansen introduced a new technique to determine the time when figs become infested with microorganisms. "In order to show the effect of maximum infestation of mites and thrips and, at the same time exclude larger insects (mainly *Carpophilus hemipterus* and *Drosophila ampelophila*) from entering the figs, the following experiment was devised. During August 10-15 the still unopened eyes of 1,557 Adriatic figs were effectively sealed by placing on the eye scales of each a small dab of Tanglefoot preparation. Such treatment did not appear to injure the fruit in any way, as it developed and matured in normal manner and season. The treated figs were allowed to mature on the trees and were not collected until they had dropped to the ground, after which they were taken to the laboratory, split open, and examined for smut and molds. As control, 400 mature figs were picked from the ground under surrounding trees and examined likewise." These figs were not cultured. Of the figs which were sealed before the eyes opened, 16.6 per cent contained visible development of molds.

*Varietal Relations.*—The fact has frequently been mentioned in the literature that there is a decided difference in the susceptibility of different varieties of figs to these diseases. In particular it has been stated, and from common knowledge may be accepted as true, that the Mission and Kadota varieties are usually immune or free from smut and mold, and souring, whereas the Adriatic and Calimyrna are commonly affected with these troubles. The reasons for this difference need further study and might throw light on the present problem. It has been commonly assumed that the lesser opening of the eye of the Mission and Kadota figs is responsible but in the light of present knowledge this explanation is not well supported. Phillips, Smith, and Smith (1925) found that Mission and Kadota figs were very susceptible to smut (*Aspergillus*) when artificially inoculated with the fungus.

*Discussion of Previous Work.*—In the basic work on the epidemiology of fig spoilage carried out by Phillips, Smith, and Smith, by Caldis, and by Hansen and Davey, several questions stand out as being of fundamental importance. Some of them are: (1) To what extent is the entrance of microorganisms into the interior of the fig dependent upon insects and what are the species concerned? (2) Is there any other mode

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<sup>5</sup> See footnote 4, page 528.

of entrance? (3) What is the importance of the eye of the fig in relation to infection? (4) When are the various spoilage microorganisms introduced into figs? The conclusions of the various workers mentioned seem to be at variance on some of these points. Phillips, Smith, and Smith, and Caldis are in essential agreement that the interior cavity of figs remains in a sterile condition until it is entered by insects; that insects

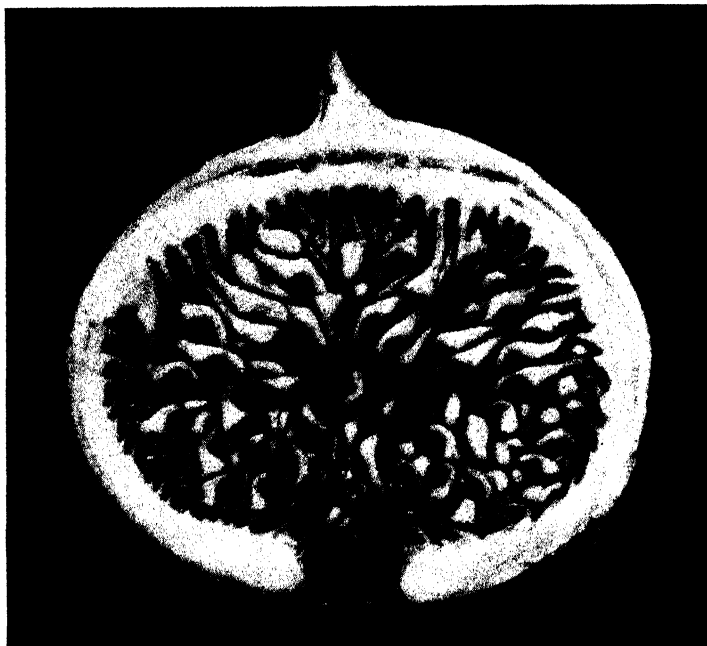


Fig. 3. Interior of nearly mature *Calimyrna* fig, twice enlarged, at the stage when ripening begins and pulp is about to soften and liquefy. It is at this stage that decay and souring begin. The whole problem of fig spoilage depends upon knowing what organisms cause this, when and how they get into the fig, and how they may be kept out or their development prevented. (From Bul. 506.)

are the principal if not the sole carriers of infection; that the dried-fruit beetle is the usual vector of the organisms which cause smut and souring, as well as of various other fungi; and that, since this insect seldom enters figs until the eye opens, the infection is not introduced until that time, previous to which the fig cavity is sterile. The fact that Caldis actually cultured 274 immature figs and found them all sterile is difficult to reconcile with some of the facts and conclusions of later workers. These workers (Hansen and Davey, 1932) conclude that "The major part of smut and mold loss is due to cryptogamic organisms carried into the green figs by predaceous mites and thrips long before

the eye scales begin to loosen," and that "The presence of *C. hemipterus* and *D. ampelophila* is not at all necessary for the occurrence of this type of spoilage."

An examination of the data and methods given by the various workers discloses several factors which might account, to some extent at least, for discrepancies in their results. Conditions may have been actually different in different seasons. Phillips, Smith, and Smith worked in 1921, Caldis in 1923, 1924, and 1925, Hansen and others in 1928, 1929, and 1930. The studies were also made in several different places. There was some difference in the variety and crop of fig studied. At least eight different kinds of figs were used by the various workers and the figs were partly of the first crop and partly of the second crop. The examination of figs for evidence of infection was made in part by the naked eye, by the microscope, and by means of cultures. In culturing the interior tissues of figs subsequent experience has emphasized the fact that the exact method of sampling the flesh is of much importance. Referring to figure 3, which illustrates the interior of a nearly mature fig in the condition in which it is cultured, two facts are of particular significance. (1) The method by which the fig is opened or split and handled may affect the possibility of contaminating the inside with organisms from the surface or atmosphere. The various investigators whose work is discussed state that the figs were "split," "opened," "cut in two" or merely that the interior was cultured. (2) The exact region or portion of the flesh which is sampled for culturing might affect the results. Whatever may be the time or method of inoculation the probability can scarcely be doubted that entrance is made through the eye of the fig. If, therefore, in one case the cultures were made from a small portion of pulp from the basal region of the cavity, farthest from the eye, and in another case from tissue near the eye or even including portions of the eye and eye scales, it may readily be seen that the results might be very different. In the previous literature most of the information on this point is vague or entirely lacking, but it is known that there was considerable variation in the methods used. It appears therefore that in order to obtain comparable results in this work a uniform or standard technique should be adopted on these and all other important details.

*New Work.*—The present work was intended to determine more comprehensively and accurately than has been attempted heretofore the occurrence of insects, mites, and cryptogamic microorganisms in figs throughout their period of development, and endeavor to explain some of the apparent discrepancies in past work. The problem was attacked in two ways: first, by following the progress of insect infestation and



cryptogamic flora in the developing fruit by observation and cultures; second, by the application of methods directed at the exclusion of insects and microorganisms from the inside of the figs.

The observations to be reported were made during 1932 almost entirely in a block of eight acres of Adriatic figs in the Tuttle district, Merced County. The trees were on heavy clay soil underlain at a depth of 2 or 3 feet by material of a more open consistency and gradually changing to sand at a depth of about 5 feet. A boring at the eastern boundary showed the water table in August, and continuously thereafter until the end of September, to be at a depth of 9 to 10 feet. Irrigations had been made in May and again on June 15. The foliage remained green and the trees appeared not to suffer to any marked extent for want of soil moisture until the crop had been harvested. Figs used in this work came almost altogether from 24 trees. The material examined for infestation, and that of which the eyes were sealed, was produced on two blocks of 9 trees, each comprising 3 trees in 3 adjacent rows in different parts of the orchard.

#### MICROORGANISMS FOUND ON CULTURING DEVELOPING FIGS IN RELATION TO INSECT INFESTATION

*Methods.*—In attempting to determine their fauna and cryptogamic flora, uncapped second-crop Adriatic figs were gathered from the trees at various stages of development for examination and culture. Since on the fig tree there is a continual formation of new fruit throughout the summer it is possible to obtain specimens of the same stages or states of maturity over a period of several weeks, after those stages have once been reached. Theoretically, therefore, the various samples of stage 1 gathered at intervals from June 20 to August 6 (table 1) would all be of the same age. The same would be true of the different batches of each of the other stages. Actually, however, it is conceivable that the later batches of each stage might contain some older, more slowly developed figs than the earlier samplings. Five more or less critical stages of development from the standpoint of disease infection were chosen. Thus stage 1 included figs in which the eye scales were tightly closed and the texture was hard. Stage 2 included figs in which the eye scales had pulled slightly apart in the growth of the fruit but still did not give an uninterrupted passage to the central cavity. Such figs while firm in texture were somewhat softer than those selected as representing stage 1. These stages correspond with those of the same numbers of Phillips, Smith, and Smith (1925) (fig. 1). Stage 3 included figs in which the eye was distinctly open providing clear access to the central cavity; at the

same time the fruit was firm and smooth in outline although yielding slightly to pressure of the thumb in picking (stages 4 and 5, figs. 1 and 2). Stage 4 included figs which presented a more or less wrinkled exterior. In these the eye was distinctly enlarged by the drying of the eye scales so that the maximum opportunity was given either insects or organisms to be carried to the interior (stage 6, fig. 2). They still retained their green color. Stage 5 included figs which had dried to a considerable extent upon the tree sufficiently to become thoroughly yellow. The later samples of stages 1, 2, and 3 were obtained from more vigorous trees in another section of the orchard owing to the lack of late figs in the area under observation. Only figs which appeared sound were taken, thus eliminating in the later stages a large number of figs which had become infected with the trouble called souring and undoubtedly reducing materially the number of figs in which causative organisms or vectors associated with that trouble were present.

The samples after being collected were taken to the field laboratory at Planada, about three miles distant, where they were examined and plated the day on which they were gathered. In making the examination each fig was first wiped off with a cloth soaked in 95 per cent alcohol. A shallow longitudinal cut was made with a sterile scalpel through the stem, and the fig split by pulling the halves apart. Examination of the interior for insects was then made by the use of a bi-objective binocular. The florets were then cut out with a sterile scalpel and placed in petri dishes. Referring to figure 3, the technique adopted as standard was to remove all the florets as completely as possible, including the tissue at the base of the eye closely enough so that an occasional eye scale from this point was taken with the sample. Upon each petri dish was recorded the kinds of infestation discovered in the examination. Melted standard potato dextrose agar was then poured from flasks over the fig tissue in each plate, endeavoring to distribute the florets as evenly as possible through the medium. It was not possible, however, in every case to distribute and submerge all the florets in the agar so perfectly as to insure positively the development of every spore or microorganism which might be present. The plates were then stored at the comparatively high summer temperature of the San Joaquin Valley until the observations were completed. Record was made of the number of insect-infested figs and of the kinds of insects observed, but not of the number of individuals in each fig. The cryptogamic organisms which developed from the figs were identified, at least as to genera, as accurately as possible, giving particular attention to those of possible pathological significance. The number of colonies was not recorded.

TABLE 1  
THE INSECT FAUNA AND CRYPTOGAMIC FLORA OF UNCAPRIFIED, SECOND CROP ADRIATIC FIGS  
AT DIFFERENT STAGES AND SEASONS

Date	Stage of fig	Number of figs	Figs with fig mite		Figs with predaceous mites		Figs with thrips		Figs with dried-fruit beetle ( <i>Carpophilus</i> )		Figs with vinegar fly ( <i>Drosophila</i> )		Sterile figs	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
June 20	1	60			3	5.0	1	1.7	0	0.0	0	0.0	55	91.7
June 21		30			3	10.0	1	3.3	0	0.0	0	0.0	30	100.0
June 27		60			23	38.4	2	3.3	0	0.0	0	0.0	54	90.0
June 28		60			15	25.0	3	5.0	0	0.0	0	0.0	59	98.3
June 29		60			17	28.3	4	6.6	0	0.0	0	0.0	51	85.0
June 30		24			7	29.2	0	0.0	0	0.0	0	0.0	21	87.5
July 5		60			24	40.0	0	0.0	0	0.0	0	0.0	45	75.0
July 6		60			28	46.7	1	1.7	0	0.0	0	0.0	25	41.7
July 7		100			36	36.0	3	3.0	0	0.0	0	0.0	38	38.0
July 8		60			18	30.0	2	3.3	0	0.0	0	0.0	25	41.7
July 9	2	35			18	51.5	0	0.0	0	0.0	0	0.0	24	68.6
July 16		60			31	51.7	0	0.0	0	0.0	0	0.0	17	28.3
July 18		60			54	90.0	1	1.7	0	0.0	0	0.0	30	50.0
July 19		60			53	88.3	1	1.7	0	0.0	0	0.0	41	68.3
July 20		58			56	96.6	1	1.7	0	0.0	0	0.0	33	56.9
July 21		39			37	95.0	0	0.0	0	0.0	0	0.0	24	61.5
July 21		60			58	96.7	0	0.0	0	0.0	0	0.0	25	41.7
Aug. 6														
Aug. 5		54			51	94.4	1	1.8	0	0.0	0	0.0	31	57.4
Aug. 25		60			37	61.7	1	1.7	1	1.7	0	0.0	28	46.7
Sept. 3	3	45			11	24.4	1	2.2	0	0.0	0	0.0	18	40.0
Sept. 16		28			3	10.7	0	0.0	0	0.0	0	0.0	8	28.6
July 21		54			53	97.2	2	3.7	0	0.0	0	0.0	16	29.6
Aug. 4		60			55	91.7	2	3.3	0	0.0	0	0.0	10	16.7
Aug. 9		60			58	96.8	1	1.7	0	0.0	0	0.0	19	31.7
Aug. 24		58			40	69.0	0	0.0	3	5.3	0	0.0	12	20.7
Sept. 3		59			8	13.5	0	0.0	0	0.0	0	0.0	8	13.6
Sept. 10		40			5	12.5	0	0.0	0	12.5	2	5.0	2	5.0
Sept. 16		27			3	11.1	0	0.0	0	0.0	0	0.0	0	0.0
Aug. 23	4	61			50	82.0	0	0.0	3	4.9	0	0.0	6	9.8
Sept. 3		56			23	41.0	0	0.0	1	1.8	1	1.8	2	3.6
Aug. 23	5	61			52	85.0	0	0.0	5	8.2	0	0.0	2	3.3

TABLE 1 (Concluded)

Date	Stage of fig	Number of figs	Figs with smut fungus ( <i>Aspergillus niger</i> )		Figs with other molds		Figs with <i>Rhizopus</i> and <i>Mucor</i>		Figs with miscellaneous fungi		Figs with souring yeasts		Figs with bacteria	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
June 20	1	60	0	0.0	2	3.3	0	0.0	3	3.3	0	0.0	2	3.3
June 21		30	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
June 27		60	1	1.7	0	0.0	1	1.7	2	3.3	0	0.0	5	8.3
June 28		60	0	0.0	0	0.0	0	0.0	1	1.7	0	0.0	0	0.0
June 29		60	3	5.0	2	3.3	2	3.3	1	1.7	0	0.0	4	6.7
June 30		24	0	0.0	2	8.3	0	0.0	0	0.0	0	0.0	1	4.2
July 5		60	4	6.7	1	1.7	0	0.0	3	5.0	0	0.0	8	13.3
July 6		60	0	0.0	0	0.0	4	6.7	0	0.0	0	0.0	27	45.0
July 7		100	2	2.0	4	4.0	0	0.0	9	9.0	0	0.0	53	53.0
July 8		60	2	3.3	4	6.7	1	1.7	7	11.7	0	0.0	23	38.3
July 9	2	35	1	2.9	2	5.7	0	0.0	1	2.9	0	0.0	4	11.4
July 16		60	0	0.0	17	28.3	1	1.7	6	10.0	0	0.0	25	41.7
July 18		60	0	0.0	7	11.7	0	0.0	11	18.3	0	0.0	13	21.7
July 19		60	1	1.7	3	5.0	0	0.0	16	26.7	0	0.0	14	23.3
July 20		58	2	3.3	3	5.2	0	0.0	10	17.2	0	0.0	10	17.2
July 21		39	0	0.0	4	10.3	0	0.0	6	15.4	0	0.0	4	10.3
July 21		60	2	3.3	17	28.3	2	3.3	8	13.3	0	0.0	10	16.7
Aug. 6														
Aug. 5		54	2	3.7	6	11.1	2	3.7	9	16.7	1	1.8	10	18.5
Aug. 25		60	2	3.3	8	13.3	0	0.0	10	16.7	1	1.7	17	28.3
Sept. 3	3	45	2	4.4	5	11.1	2	4.4	16	35.6	0	0.0	10	22.2
Sept. 16		28	2	7.1	2	7.1	0	0.0	17	60.7	0	0.0	2	7.1
July 21		54	0	0.0	7	13.0	4	7.4	19	35.2	0	0.0	19	35.2
Aug. 4		60	10	16.7	13	21.6	12	20.0	7	11.7	0	0.0	16	26.6
Aug. 9		60	5	8.3	15	25.0	7	11.7	12	20.0	0	0.0	19	31.7
Aug. 24		58	20	34.5	10	17.2	2	3.3	13	22.4	7	12.1	8	13.8
Sept. 3		39	20	51.3	5	12.8	13	33.3	14	35.9	3	7.7	9	23.1
Sept. 10		40	18	45.0	16	40.0	2	5.0	7	17.5	6	15.0	12	30.0
Sept. 16		27	4	14.8	5	18.5	1	3.7	5	18.5	14	51.8	13	48.2
Aug. 23	4	61	32	52.5	5	8.2	16	26.2	9	14.8	5	8.2	7	11.5
Sept. 3		56	34	60.7	10	17.9	10	17.9	8	14.3	7	12.5	11	19.6
Aug. 23	5	61	42	69.0	2	3.3	18	29.5	3	4.9	9	14.8	9	14.8

*Results.*—Under the conditions described the results cannot be expected to be of absolute accuracy. Most of the insects recorded are of a free-moving character and some might easily have entered and left the figs before the observations were made. Others are small and a few individuals might have been overlooked, especially since it was necessary to manipulate the fig as little as possible on account of the subsequent culturing. Although no record was attempted of the number of individual insects in each fig, it was evident that, in the case of predaceous mites especially, there was much variation in this respect. This may have affected the degree of infection with microorganisms. Since each fig before culturing had to be split open and examined for insects in the open laboratory a considerable chance of contamination could not be avoided and it is probable that this sometimes occurred. Since, however, figs of different stages were usually being cultured simultaneously a check on air-borne contamination was provided and it is not believed that this was a serious source of error. To a considerable extent also the characteristic flora of the figs was different from that of the room. As a rule it is probable that the results for microorganisms were too low rather than too high, for the reason noted above relating to the difficulty of thoroughly distributing and submerging the fig material in the culture medium. This would apply especially in the early part of the season when the number of spores per fig was small. It is to be expected, however, that determinations made by culturing the figs will be higher and reveal more kinds of organisms than those made by simply examining the figs with the eye or microscope. Many spores or latent infections might be present in the figs which would never develop under natural conditions but come to light only in the culture plate. Table 1 gives the data of the entire experiment with the number and percentage of figs in each batch found infested with insects (which are listed in separate columns) and the same data for the cryptogamic flora. No attempt was made to record the amount or location of contamination in individual figs.

The figures under "Figs with fig mite" indicate that these were abundantly present in figs of all stages from the beginning of the observations up to the latter part of August. A large percentage of the figs examined from mid-July to the end of August showed extensive damage to the florets as a result of this infestation. From then on the mites noticeably decreased in figs of all 5 stages, indicating possibly that they begin to leave the fruit at that season.

"Predaceous mites," so far as the data show, were comparatively scarce in the figs until late in June but by September 1 were present in practically all green figs. As the figs ripened these mites apparently

left them. According to notes not included in the table it may be stated that early in June the predaceous mites were present in the fruit in much lesser numbers as well as in a smaller percentage of the figs than later in the season. In the earlier cases often only a single mite or at most only a few were found per fig, while later, when the eyes opened, a pronounced increase was apparent both in the number of figs infested and the number of predaceous mites found in each fig. The mites were of two or more species but no attempt was made to identify them or record their relative abundance. Smith and Hansen (1931) give some information regarding the species of such mites found in figs.

Thrips were not found abundant in figs at any time during the season. Compared with the figures of Hansen (1929) for 1928 and 1929, and those of Hansen and Davey (1932) for 1930, it appears that there is a wide variation in the occurrence of thrips in figs in different places and seasons. Here again, as with predaceous mites, several different species were concerned but their identity or relative abundance was not determined. Hansen (1929) and Smith and Hansen (1931) discuss this, mentioning six different species of thrips found in figs. The few cases of thrips found in this work were scattered throughout most of the season. Fewer thrips were observed in figs the latter part, which appears to be the rule except in the case of one species, the black thrips of bean and cotton (*Heliothrips fasciatus* Perg.). This species is often found very abundantly in green figs late in the fall.

The dried-fruit beetle was never found abundantly, but all the infestation occurred after August 20 and only in figs in which the eye was open. The same was true of the vinegar fly, which occurred even less frequently. Both the beetle and fly may have passed into and out of some of the figs without being observed or recorded. The fact also that only sound figs were examined, those showing signs of spoilage being rejected, must have eliminated many which contained or had been entered by beetles and vinegar flies.

The data under the heading, "Sterile figs" represent only figs which gave no growth in agar plate cultures containing most of the interior portion of the fig. Although these figures show considerable fluctuation (due partly perhaps to difficulties of technique) they seem to display certain well-marked trends. In general the figs (stage 1) cultured during the month of June appeared to be nearly all sterile, after which figs with closed eyes (stage 1 and stage 2 in part) showed an average contamination with microorganisms of about 50 per cent. Of the open-eye figs of stage 3, about 25 per cent remained sterile until September, after which nearly all developed a cryptogamic flora.

The figures given under the heading "Figs with smut fungus" show a small, scattering but definite occurrence of *Aspergillus niger* in sound, closed figs of stage 1 from the beginning of the observations. Of the 946 figs which were cultured less than 2 per cent developed this fungus. In 8 of the 17 batches all of the figs were free from the smut fungus and only one fig with *Aspergillus* was found in each of three other lots. In stage 2 (eyes commencing to open) the smut fungus was found in a small but rather uniformly increasing percentage of the figs. In stage 3 (eyes open) smut showed a very marked increase, becoming even more pronounced in stages 4 and 5, where more than 50 per cent of the figs contained this fungus. These, it may be repeated, were all selected, sound figs which showed little if any smut development.

Under "Figs with other molds" are grouped together the fungi which cause visibly moldy figs (*Alternaria*, *Cladosporium*, *Hormodendrum*, *Macrosporium*, and *Penicillium*). The situation here showed an almost total absence of these fungi from figs during most of June, a small percentage of contaminated figs during the first part of July, and a pronounced increase occurring about the middle of July. The most mature figs up to this time were all of stage 1, having tightly closed eyes. After July 16 the percentage of figs containing these mold fungi did not increase significantly in figs of any stage, even those with open eyes.

"Figs with *Rhizopus* and *Mucor*" showed a very light and scattering occurrence previous to the opening of the eye, a marked increase at that time (stage 3) and some further development in the later stages.

Under "Figs with miscellaneous fungi" is included a variety of forms which have no known significance in fig spoilage. Most of them are of the yeastlike fungi referred to in the footnote on page 528, together with species of *Acrostalagmus*. These forms constitute a rather characteristic flora in green figs. The data show that the miscellaneous fungi were present very early in a considerable percentage of green, closed figs and that the percentage increased after the opening of the eyes.

"Figs with souring yeasts" includes forms of *Mycoderma*, *Pseudo-saccharomyces*, *Hansenia*, and *Pichia* which were found associated with typical fig souring. Such yeasts were entirely absent from all the figs of stage 1 and the first three batches of stage 3. Two figs of stage 2 were found to contain souring yeasts. About the middle of August these organisms began to appear commonly in figs of stage 3 and, in the last batch examined on September 16, were found in over 50 per cent of the fruit. No figs showing visible souring were included in the samples.

The figures for "Figs with bacteria" include several types which were fairly constant in most of the cultures but which, so far as known, are of

no primary importance in fig spoilage. These organisms appeared to be the first to invade green figs. They were present in 50 per cent of figs of stage 1 soon after July 1, following the earlier period when most of the figs were sterile.

*Conclusions and Correlations from Examination and Culturing of Figs.*—The results reported in table 1 suggest some fairly definite conclusions as to the epidemiology of fig-spoilage diseases. The high percentage of sterile figs found during June indicates that there was a period at that time of year when the interior of young, closed figs was comparatively free from microorganisms. Gradually, however, contamination took place until, after July 1, less than 50 per cent of figs in the same stage of development (stage 1) were sterile. Figs of stage 1 continued in about this degree of contamination throughout the season. The earliest contamination consisted of a rather specific flora of bacteria, yeastlike fungi, and certain other miscellaneous fungi which never cause visible injury to figs. Regarding insect vectors of this early contamination three possible agents may be considered: fig mites, predaceous mites, and thrips. The fact that figs of stage 1 (eye tightly closed) were nearly all sterile during June and at the same time all heavily infested with the fig mite, confirms previous conclusions that this mite is not a carrier of microorganisms (Phillips, Smith, and Smith, 1925, p. 32; Smith and Hansen, 1931, p. 28). "Predaceous mites," so far as these data show, were present in very few figs when the first two batches of stage 1 were examined, but the percentage of infestation materially increased during the period (June) when most of these figs of stage 1 were still sterile. Of the 60 figs examined on June 27, for example, 23, or 38.4 per cent, were infested with predaceous mites; but 54, or 90 per cent of the same figs were sterile. On June 28, 60 similar figs were examined and 15, or 25 per cent, showed predaceous mites, yet all but one (98.3 per cent) were sterile. The mites in individual figs were fewer in number at this time than later and it may be true also that they had not as yet penetrated the interior of the figs very extensively. Attention may also be directed to the showing of Hansen and Davey (1932) that of 226 predaceous mites cultured by them, 122, or 43.5 per cent, were free from cryptogamic organisms. It is not unlikely that the percentage of infection of the mites, or in other words the abundance of mold spores, may be less in the earlier part of the season. Unless some of these factors are significant it is difficult to reconcile the low percentage of cryptogamic infection in figs of stage 1 in June and the high infestation with predaceous mites at the same time, with the idea that these mites are important vectors of spoilage organisms. Thrips



were present in such small and irregular numbers that no conclusions could be drawn as to their importance.

The next striking development was the marked increase in molds which took place in closed figs (stage 1) after July 15. No correlation of this with any insect vector is apparent, unless it be the increased abundance of predaceous mites in individual figs, a difference in the species present, increase in their cryptogamic contamination, or more extensive penetration of the inside of the figs.

The small amount of smut present in figs of stages 1 and 2 might be correlated with predaceous mites or, to a slight extent, with thrips. The large and sudden increase of *Aspergillus* and *Rhizopus* which occurred early in August in figs of stage 3, followed closely by the development of souring, correlates with the time of the opening of the eye of the fig, the ripening of the first figs, and the appearance in numbers of the dried-fruit beetle. No evidence is afforded, however, as to whether there was any connection between these events or if it was merely a coincidence. Vinegar flies were not sufficiently abundant to justify any conclusions. The conclusion of previous workers that thrips, predaceous mites, and fig mites are not vectors of souring yeasts is supported by the results given in this table. The nonsouring yeasts or yeastlike fungi previously mentioned by Hansen, by Smith and Hansen, and by Hansen and Davey as being carried by thrips are included here under "Miscellaneous fungi." Of the true souring yeasts (*Mycoderma*, *Pseudosaccharomyces*, *Hansenia*, *Pichia*) not a single colony developed from the 946 figs of stage 1 in which fig mites and predaceous mites were abundant.

The older figs of stages 4 and 5 are probably of no additional significance. At the latter stage the fig commences to dry, the infection period is passed, and the high concentration of sugar in the fig makes it no longer a favorable medium for the growth of microorganisms, or for the insects which attack green figs. To such causes, and the fact that all figs showing spoilage were rejected, is probably due the apparent decrease of infection in the later stages.

## EXPERIMENTS IN EXCLUDING INSECTS AND MICROORGANISMS

*Sealing the Eyes of Figs.*—The use of Tanglefoot to seal the eyes of figs has already been mentioned (Hansen and Davey, 1932). In the present work this method was used on four trees and on a much larger number of figs than before. The sealing was carried out at two periods: figs with closed eyes on two trees were sealed between June 22 and June 25, and those on two other trees between July 28 and August 4, hoping at the early period to forestall the infestation by predaceous mites. Examination of green figs at about the first time of sealing, however, indicated that a considerable percentage was already infested (see table 1) by the time the sealing was completed on June 25. The figs were allowed to mature and fall, and were gathered from the ground about once a week. A number of unsealed figs from the same trees, together with the crop from two adjacent trees were picked up at the time by way of controls. All of these trees, as described on page 532, were adjacent to those from which the figs were taken for culturing (table 1). In making the examinations of the figs and in reporting results, all with broken seals were carefully segregated and reported separately. The figs were not cultured but simply examined for spoilage. Consequently most of the miscellaneous fungi and all of the bacteria reported in table 1, as determined by culturing the figs, would fail to be detected in these figs. The percentages of smut, *Rhizopus*, and other molds would also be expected to be lower here than in the experiments where the figs were cultured. In the latter case the presence of a few spores would be responsible for a record of the fungus, while, in the method used here, visible growth and spoilage in the fig was required. In so far as simple, microscopic examination of the growth within the fig could determine, the molds were assigned to the same groups as in table 1.

*Results.*—The results obtained in this experiment are presented in table 2. The tables gives the figures for the crop of each tree, considering separately the figs in which seals were intact, those with broken seals, those on the same trees not sealed, and figs from adjacent trees on which none were sealed. In regard to smut it will be noted that on all the trees the percentages in the unsealed figs were very much greater than in the fruit which had been sealed. On trees 1 and 2 on which the figs were sealed early, less than 2 per cent of the figs with unbroken seals developed smut, while the average of unsealed figs was more than 10 per cent. Similar figs sealed later on trees 3 and 4 averaged about 3 per

TABLE 2  
RESULTS OF SEALING EYES OF FIGS PREVIOUS TO OPENING

Tree number	Condition of seal when examined	Number of figs	Date of sealing	Figs with smut fungus ( <i>Aspergillus niger</i> )		Figs with other molds		Figs with <i>Rhizopus</i> and <i>Mucor</i>		Figs with souring	
				Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1	Intact.....	2,108	June 22-25.....	32	1.5	321	15.2	2	0.1	0	0.00
1	Broken.....	1,233	June 22-25.....	26	2.1	63	5.0	1	0.1	0	0.00
1	Not sealed.....	517	Not sealed.....	70	13.5	32	6.0	..	..	0	0.00
5	Not sealed.....	2,322	Not sealed.....	219	10.6	92	4.0	29	1.3	5	0.20
2	Intact.....	1,730	June 22-25.....	32	1.8	344	20.0	3	0.2	0	0.00
2	Broken.....	1,668	June 22-25.....	48	2.9	177	10.6	0	0.0	0	0.00
2	Not sealed.....	358	Not sealed.....	24	6.7	12	3.3	..	..	0	0.00
5	Not sealed.....	2,322	Not sealed.....	219	10.6	92	4.0	29	1.3	5	0.20
3	Intact.....	1,031	July 28-Aug. 4.....	36	3.5	103	10.0	4	0.4	0	0.00
3	Broken.....	274	July 28-Aug. 4.....	6	2.2	33	12.0	1	0.4	0	0.00
3	Not sealed.....	908	Not sealed.....	124	13.6	83	9.2	..	..	2	0.20
6	Not sealed.....	4,053	Not sealed.....	277	6.8	79	2.0	35	0.86	6	0.15
4	Intact.....	1,180	July 28-Aug. 4.....	31	2.6	78	6.6	2	0.2	0	0.00
4	Broken.....	240	July 28-Aug. 4.....	7	2.9	14	6.0	0	0.0	0	0.00
4	Not sealed.....	1,468	Not sealed.....	72	4.9	36	2.5	..	..	4	..
6	Not sealed.....	4,053	Not sealed.....	277	6.8	79	2.0	35	0.86	6	0.15

cent smut while the percentage in the unsealed was much higher. These results indicate that there was a small and slowly increasing percentage of smut infection in the figs before the eyes opened, but that the great bulk of the infection entered after the opening of the eyes. In the case of other molds, on the contrary, it appears that the maximum amount of infection took place early in the development of the fruit before the opening of the eye, and could have had no relation to the entrance or exclusion of insects as large as the dried-fruit beetle. In fact, the sealed

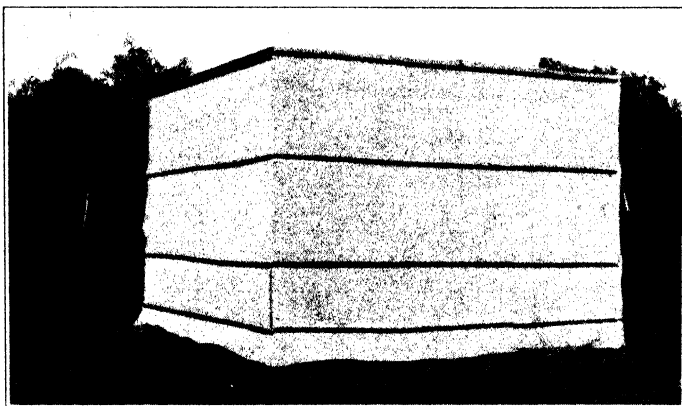


Fig. 4. Tent over fig tree to exclude insects.

figs show a decidedly greater percentage of spoilage by mold than those with open eyes. This may have been due to increased humidity within the fig. *Rhizopus* was present in very small amounts in these figs, both sealed and unsealed. No souring took place in the figs with sealed eyes and only a very few of the control figs were sour.

*Screening of Trees.*—In 1922 an experiment was undertaken by Phillips, Smith, and Smith (1925) in which two large Adriatic fig trees were screened from infestation by the dried-fruit beetle by tenting them over with unbleached muslin. Such a measure was effective in keeping out the insect but a large amount of molding occurred on the exterior of the fruit. Dried-fruit beetles were introduced into one of the tents but apparently failed to enter the fruit upon the tree.

In 1932 a similar experiment was conducted in the present investigation. The exterior molding of the fruit was avoided by permitting freer air movement through the selection of smaller trees and the use of more open material as a screen. Six trees were selected. Four of these were completely enclosed on sides and top with a screen (fig. 4) of absorbent gauze (cheesecloth) which had a denseness of weave of 14 threads to

TABLE 3  
RESULTS OF SCREENING FIG TREES TO CONTROL DRIED-FRUIT BEETLES

Tree number	Type of screening	Beetles introduced	Number of figs	Figs with smut fungus ( <i>Aspergillus niger</i> )		Figs with other molds		Figs with souring		Figs with beetles	
				Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
7	Complete	No	753	24	3.2	21	2.8	0	0.00	0	0.0
8	Complete	No	632	34	5.4	26	4.1	0	0.00	0	0.0
9	Complete	No	869	86	9.9	36	4.1	0	0.00	0	0.0
10	Complete	Yes	688	28	4.1	28	4.1	10	1.50	11	1.6
11	Open top	No	1,037	102	9.4	53	5.0	10	0.90	8	0.7
12	Open top	No	808	51	6.3	41	5.2	0	0.00	0	0.0
5	None	No	2,322	219	10.6	92	4.0	5	0.20	0	0.0
6	None	No	4,053	277	6.8	79	2.0	6	0.15		

TABLE 4  
THE RELATION OF ANTS TO FIG SPOILAGE

Number of trees	Number of figs	Infestation with ants	Figs with smut fungus ( <i>Aspergillus niger</i> )		Figs with <i>Rhizopus</i>		Figs with other molds		Figs with souring	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
8	800	Infested Not infested	42	5.2	33	4.1	43	5.3	18	2.2
8	800		45	5.6	32	4.0	58	7.2	15	1.6

the inch. A floor of unbleached muslin was provided and fitted closely around the tree trunk, the whole structure being made as tight as possible. The dimensions of these tents were: sides 12 feet, height 9 feet. Two other trees were similarly screened except that no top was provided; the sides were 12 feet in height. All figs approaching ripeness were removed before screening with the object of precluding any chance of infestation with dried-fruit beetles. The construction of the screens was completed between July 30 and August 2. On August 11, 70 sour figs containing adult beetles and larvae were placed in a shallow granite dish within a 50-pound lug box. The lug box contained a layer of soil 2 to 3 inches deep, was loosely covered by brown wrapping paper, and the whole introduced within the screen over one tree. The crop on the trees was allowed to drop and was not gathered until August 21, when the figs were removed from the floor and the trees were stripped of any fruit on the branches.

*Results.*—Table 3 shows the numbers of smutty and moldy figs, sour figs, and figs infested with the dried-fruit beetle in the crop from each tree, including the two adjacent unscreened trees (Nos. 5 and 6) which have already appeared in table 2. It will be seen that the percentage of smut in the figs from screened trees, which are believed to have been entirely free from dried-fruit beetles, ranged as high as in those of unscreened trees; and the screened tree into which beetles were introduced ranked next to the lowest in amount of smut. The amount of mold also showed no relation to the presence or absence of beetles. Souring occurred on screened trees only where beetles were present within the screen. On such trees beetles were discovered in both normal immature figs and figs already sour.

*Ants as Carriers of Fig-Spoilage Organisms.*—The presence of black ants in numbers on fig trees drew attention to them as possible carriers of fruit-spoilage organisms. It was noted that many trees were badly infested with ants while others were apparently free from such infestation. The crops of 8 infested and 8 uninfested trees were therefore sampled, 100 mature figs being taken from the ground beneath each tree. Table 4 gives the results of this experiment.

*Results.*—The figures show that spoilage troubles were almost identical in the crops from ant-infested and noninfested trees and that the dissemination of these troubles can probably therefore not be attributed to this agency.

## DISCUSSION

The results obtained by the three different lines of attack upon the problem of the epidemiology of fig spoilage are essentially in agreement and suggest a number of conclusions in relation to previous work and ideas on this subject.

*Sterility of Immature Figs.*—The former conception of the epidemiology of fig spoilage was based largely upon the following hypotheses, first formulated by Phillips, Smith, and Smith (1925), that (1) "In the climate of the San Joaquin Valley, the interior cavity of Adriatic figs usually remains sterile until it has been entered by insects." (2) "The smut fungus is usually carried into figs by insects, of which the dried-fruit beetle, *Carpophilus hemipterus* (Linn.) appears to be the most important." (3) "Indications point to the dried-fruit beetle as being also an important carrier of some other forms of decay." (4) "Figs become infected with smut when they are still on the tree, just at the time when the eye opens and the fruit begins to soften." The work of Caldis (1927), (1930), supported this point of view. Subsequently, the work of Hansen (1929), Smith and Hansen (1931), and Hansen and Davey (1932) established the fact that a considerable percentage of the figs which they examined were not sterile previous to the opening of the eye and presumably could not have been inoculated with organisms introduced by insects as large as the dried-fruit beetle. These investigators directed attention to thrips and predaceous mites as possible vectors.

The present work establishes very plainly the fact that many figs are not sterile just previous to the opening of the eye, and that closed figs may gradually become contaminated during the growing season. In table 1, for example, the 946 figs which reached stage 1 and were examined and cultured at intervals during June and July indicate a gradual progress from 100 per cent sterile in those which were of this stage of maturity early in June, to less than 50 per cent in those which reached the same stage of development about August 1. It is noticeable that much of this early contamination, and practically all of it previous to about July 15, was due to bacteria and the organisms listed as miscellaneous. This flora was a characteristic one and did not indicate haphazard, air-borne contamination. As to vectors, it may be assumed that the figs of stage 1, table 1, had not been entered by dried-fruit beetles, and the same is nearly as positive concerning the figs with sealed eyes, in table 2, and those in the enclosed cages, in table 3. It therefore seems

safe to conclude that *Carpophilus* is not the sole carrier of cryptogamic infection and that if a living vector is involved it must be of sufficiently small size to penetrate the closed eye of the fig. According to the data in table 1 the fig mite (*Eriophyes*), and various species of predaceous mites and thrips are possibilities in this connection. The fig mite as a possible carrier has already been practically eliminated by previous work of others and by the data in table 1 where figs 100 per cent heavily infested with fig mite were practically all sterile. Thrips cannot be excluded as potential carriers of infection, but during the season when this work was done they did not seem abundant enough (table 1) to account for much of the cryptogamic flora. Predaceous mites seem then to be the only remaining possibility, since, with the amount of work which has been done upon the fauna of green figs, it is doubtful if any other important vector has been overlooked. Allusion has already been made (page 539) to the situation which was found (table 1) in figs of stage 1 near the end of June, when almost all were sterile as to cryptogamic flora and yet from 25 to 40 per cent were infested with predaceous mites. Although a plausible explanation of this condition has been suggested, it is still evident that final and complete proof has not yet been established regarding insect transmission of fig-inhabiting micro-organisms.

*Smut*.—In this work it seems to be clearly shown that the idea of the dried-fruit beetle's being the sole vector of fig smut (*Aspergillus niger*) is no longer tenable. Table 1 shows definitely that a small percentage of figs which had never been entered by this or any other comparatively large insect contained the spores of this fungus.<sup>6</sup> From this table it appears, however, that the percentage of figs entered by the smut fungus was very low until after the fruit had reached that stage of maturity when the eyes open and ripening began, whereupon a very large increase in the percentage of invasion took place. The coincidence of this with the entrance of the beetle naturally suggests a connection between the two events and from the data in table 1 alone it might easily be concluded that *Carpophilus* is the principal vector of smut. Table 3, however, seems to show very plainly that when the beetle was entirely excluded from figs by screening the percentage of smut developed was just as great as in fruit which was fully exposed to this insect. If it be assumed that the possibility of the beetle as a primary carrier of smut

<sup>6</sup> One fig of stage 2, in which the eye was still practically closed, contained a beetle, and Smith and Phillips (1922) record finding a few cases of ants, beetles, and flies in green closed figs. These cases are too exceptional to seem of any significance in the present connection.



is eliminated by these experiments, then the fact of the presence of *Aspergillus* spores in closed-eye figs may be looked upon as a similar situation to that which has just been discussed in regard to miscellaneous fungi. Predaceous mites seem to be the only carriers which could have been extensively involved but it cannot yet be said that the case against them is a complete one. It is not entirely impossible that the small numbers of thrips present in closed figs might have had some significance regarding this early smut infestation. The fact of the increase of *Aspergillus* in figs with open eyes (tables 1 and 2), taken with the showing in table 3 that excluding the *Carpophilus* beetle did not prevent this, might be taken to suggest that the opening of the eye provides access to the interior of the fig for large numbers of *Aspergillus* spores from the atmosphere. It is, however, also true that the population of predaceous mites and fig mites and the injury to fig tissue by the latter are all at their height at this time and other complicating factors no doubt exist. Further work is needed upon this important point. Phillips, Smith, and Smith (1925) reported negative results from their limited experiments on air-borne infection. It should of course be remembered that the figures in table 1 are based on cultures and represent figs all of which appeared to be sound and free from visible smut, while the figs listed under "Smut" in tables 2 and 3 showed visible development of the disease. There is considerable uncertainty, therefore, as to how much significance the late-entering, abundant invasion of open-eye figs by *Aspergillus* spores has in the development of visible smut or commercial spoilage of this type.

*Other Molds.*—In the figs from which the figures presented in table 1 were obtained it is fairly definite that the fungi which cause what is commonly called "mold" (*Alternaria*, *Hormodendrum*, *Cladosporium*, etc.) became abundant in closed figs of stage 1 about the middle of July, but were not present during the first part of the season in figs which had reached stage 1 at that time. No marked increase took place after the eyes opened. Sealing the eyes or screening the trees (tables 2 and 3) did not affect development of mold except that sealing increased it somewhat. The question of insect transmission or the method of the introduction of these molds into figs with closed eyes has the same aspects as in the case of other fungi. Predaceous mites and the few thrips which were present seem to be the only possible vectors but further proof is needed in regard to them.

*Souring.*—The figures given in table 1 corroborate the conclusion of previous workers that souring yeasts do not enter the fig until after the eye opens and that this time coincides with the appearance of the dried-

fruit beetle. This, however, does not give proof that these facts have any relation to each other. Tables 2 and 3 indicate that when beetles were excluded, either by sealing or screening, no souring occurred, whereas a certain amount developed in beetle-infested figs. The data are too meager, however, to be taken as final. The results coincide with those of Phillips, Smith, and Smith (1925) and those of Caldis (1930).

## SUMMARY

The epidemiology of spoilage diseases of uncapped, second-crop Adriatic figs was studied at Merced, California, by three different methods with particular reference to insect transmission.

The internal fauna of developing figs was found to consist of the fig mite, *Eriophyes fici* Ewing, various species of predaceous mites, various species of thrips, the dried-fruit beetle (*Carpophilus hemipterus* L.), and the vinegar fly (*Drosophila ampelophila* Loew.). The last two mentioned were found to enter the figs only after the eyes had opened; the others were found throughout the season in immature figs with the eyes still closed. Ants were also found to infest ripening figs in some cases.

Up to about July first, green, nearly full-grown figs with closed eyes (stage 1) were found to be nearly all internally sterile. In the successively developing figs which reached this stage after that time an increasing percentage was found to contain cryptogamic microorganisms.

The earliest flora to appear in figs of stage 1 consisted mostly of bacteria and certain yeastlike fungi.

In figs of stage 1 the fungi which cause moldy figs (*Alternaria*, *Hormodendrum*, *Cladosporium*, etc.) were first found abundantly in fruit which reached that stage about July 15. No marked increase thereafter in the percentage of figs of any stage infected with these fungi was found.

The fig-smut fungus (*Aspergillus niger*) was found to be present in a small percentage of figs previous to the opening of the eye. After the eyes opened this percentage was much increased.

The yeasts which cause fig souring were found to be entirely absent from figs until after the eyes had commenced to open.

No relation could be seen between the presence in figs of the fig mite (*Eriophyes fici*) and any type of infection.

Evidence was obtained that the dried-fruit beetle (*Carpophilus*) is not an important factor in the transmission of smut and mold, but may be the principal carrier of the yeasts associated with souring. During the season when this work was done thrips were not present in figs in sufficient numbers to warrant any final conclusions as to their importance as carriers of infection.

Predaceous mites and, to a much less extent, thrips, were the only living vectors to which the transmission of smut and mold could be attributed.

No relation was found between the activities of ants in figs and the spread of smut and mold, and souring.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

AUGUST, 1933

No. 14

## EFFECT OF COVERCROPS ON THE SOIL SOLUTION AT DIFFERENT DEPTHS UNDER ORCHARD CONDITIONS<sup>1</sup>

E. L. PROEBSTING<sup>2</sup>

Two earlier papers<sup>(4, 5)</sup> have presented progress reports concerning changes in concentration of the more important ions in the soil solution under a variety of covercrop treatments and with different species of trees. These results were obtained from the orchard of the Pomology Division of the California Agricultural Experiment Station at Davis. The crop history has been given<sup>(4)</sup> in a former paper and is not essential for consideration of the data given here. The plot treatments were alfalfa sod (*Medicago sativa*); a summer covercrop of mat bean (*Phaseolus aconitifolius*), which was superseded by *Dolichos lablab* in the seasons 1931 and 1932; and winter covercrops of rye (*Secale cereale*) and of *Melilotus indica*. These were all compared with three clean-cultivated checks. The treatments were duplicated. They ran in strips across the species plantings of pears, prunes, apples, Japanese plums, cherries, apricots, and peaches as shown in figure 1. The method used in obtaining the soil solution has been described elsewhere<sup>(3)</sup>, and is essentially a displacement rather than an extraction with an excess of water:

In the preceding reports, the data have been based on analyses from composite samples of the upper 4 feet of soil. Because many roots penetrate to greater depths, analyses have been made of the soil solution obtained to a depth of 8 feet. The present report shows the results of four composite samples of 2 feet each to a total depth of 8 feet. This changed procedure has reduced the number of plots that could be

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sampled in a given period to one-fourth of the previous number. The time interval between samples of a given plot has therefore been markedly increased, and the number of points on the graph of a season's results correspondingly reduced. The seasonal sequences have been so

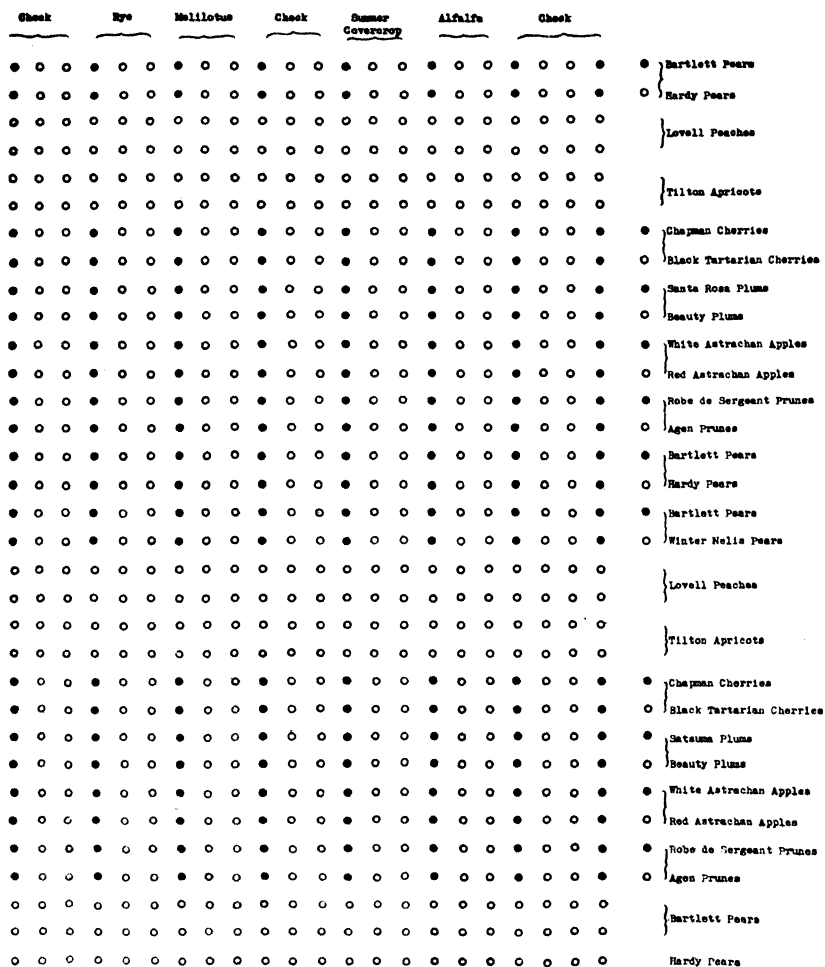


Fig. 1. Planting plan of covercrop experiment. Block A is the lower 17 rows; block B the upper 16. Numbering begins at the lower right-hand corner in each block. Symbols indicating the kinds of fruit trees in each row are given at the right.

regular, however, that there is probably no serious objection to the changed method of sampling. The first samples taken at the greater depth were secured in 1929 in four plots of block B, namely the north check and alfalfa plots of pears and peaches. These samples showed

such striking differences that the method was extended to 28 plots for each of the past three seasons. Sampling of the peach and pear plots was continued until June, 1931, and then a change was made to the adjacent rows of apricots and prunes. The reason for this change was primarily that a very large number of holes had been made in limited areas, cutting many roots and thus causing an increasing heterogeneity in the plot. Areas tapped by roots lose moisture and solutes, and when the roots are cut a new situation develops. Until new roots grow into such a region, the area is not typical of the plot in question. The two fruits chosen, besides lying adjacent to the fruits already used, had the additional advantage of differing from each other in growth and fruiting habits as well as in handling. The apricot tree is much larger and, probably, deeper rooted than the prune. It matures its fruit early in the season, being harvested in June. It is pruned severely, and the fruit is thinned. The prune, in contrast, matures its fruit in September, is pruned very little, and is rarely thinned.

These differences in behavior were expected to influence somewhat the character of withdrawals made on the soil solution at various periods of the year. In the course of these investigations, it was found that there was a marked tendency for the soil solution under apricots to resemble that under peaches, and for that under prunes to approximate that under pears. Few data have been secured on the other three species, namely, apples, Japanese plums, and cherries, and these will not be considered in any of the discussion.

In order to conserve space in the presentation of data, the major results of only one season, 1930, will be reported. Important deviations from these typical cases will be noted where they occur. The large number of solutions analyzed (over 1,200 in the period under discussion) would seem to justify the withholding of a considerable portion of the data.

## NITRATE

It has been pointed out<sup>(4, 5)</sup> that in the upper 4 feet of soil the nitrate concentration varies seasonally, usually showing its minimum in the spring and its maximum in the fall; that alfalfa reduces the  $\text{NO}_3$  concentration, as do peaches to a lesser degree. The data presented here (tables 1 to 4, inclusive), support these conclusions. In addition, a number of interesting relationships can be seen. Perhaps the most striking is the contrast between alfalfa and check plots. The divergence in concentration between these two series of plots is greater below 4



feet than above that depth. The relation between nitrates under peaches and those under pears, however, is the reverse at the lower depths of that in the top 4 feet. That is, the concentration of nitrate is greater under peaches than under pears at the lower depth.

TABLE 1

NITRATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK A,\*  
IN PARTS PER MILLION OF NO<sub>3</sub> OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	{ May 23.....	230	270	1,490	2,240
	{ July 2.....	240	140	430	1,100
	{ September 12.....	380	430	1,950	1,720
	{ December 23.....	460	210	1,030	1,390
Alfalfa sod	{ May 26.....	570	220	100	160
	{ July 7.....	540	180	90	140
	{ September 18.....	120	200	70	60
Summer covercrop	{ May 27.....	170	130	210	1,040
	{ July 8.....	300	120	170	760
	{ September 14.....	210	100	210	230
Clean-cultivated check	{ May 28.....	160	130	660	1,800
	{ July 9.....	270	340	.....	1,370
	{ September 19.....	240	80	1,100	1,960
Winter covercrop of melilotus	{ May 29.....	240	60	1,110	1,750
	{ July 10.....	.....	70	730	1,570
	{ September 20.....	280	80	350	1,200
Winter covercrop of rye	{ May 30.....	220	60	270	510
	{ July 11.....	460	70	160	560
	{ September 25.....	420	80	170	450
Clean-cultivated check	{ June 2.....	200	130	630	1,840
	{ July 15.....	.....	100	710	1,200
	{ September 27.....	430	160	1,100	2,040

\* Block A consists of trees planted in 1922; block B of trees planted in 1923.

According to studies by Beckett and Huberty,<sup>(2)</sup> alfalfa roots may be fairly well distributed at depths to 8 feet under the conditions prevailing at Davis. Probably, therefore, the alfalfa plant has reduced the nitrate concentration to a depth of at least 8 feet by direct absorption.

The alfalfa was plowed up in the fall of 1929 because weeds had become established in these plots. In the top 2 feet, the NO<sub>3</sub> concentration rose steadily for a year, beginning within a month after plowing and reaching a maximum the following autumn. The lower depths showed progressively less effect than the surface, there being no significant

increase at 6 to 8 feet. When the plots were reseeded in the fall of 1930, the  $\text{NO}_3$  concentration dropped rapidly again; and it has since been maintained at a low level in all plots. The alfalfa seems to be the major

TABLE 2  
NITRATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK B,  
IN PARTS PER MILLION OF  $\text{NO}_3$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	March 21	260	170	510	900
	May 5	210	170	440	970
	June 10	200	150	450	970
	August 12	280	230	630	930
	November 7	470	270	670	620
Alfalfa	March 1	260	70	30	30
	May 6	590	150	50	30
	June 19	380	130	100	60
	August 13	190	150	240	40
	November 8	530	120	30	30
Summer covercrop	May 7	130	140	640	730
	June 18	270	100	310	1,000
	August 14	230	170	660	1,120
	November 14	350	180	900	1,040
Clean-cultivated check	May 8	150	200	640	870
	June 17	190	90	530	1,080
	August 15	120	110	590	730
	November 23	360	130	660	1,350
Winter covercrop of melilotus	May 9	130	70	320	630
	June 16	210	80	130	520
	August 16	100	50	100	360
	November 25	60	70	180	600
Winter covercrop of rye	May 12	150	80	70	90
	June 13	210	90	70	120
	August 21	280	80	90	200
	November 29	360	80	80	230
Clean-cultivated check	May 13	210	230	300	1,040
	June 12	160	80	280	1,100
	August 22	210	100	410	1,090
	December 2	460	70	340	600

factor affecting the level of  $\text{NO}_3$  concentration in plots having this treatment. Differences between species of trees are obscured by the greater effect of the alfalfa.

The effect of the summer covercrop on nitrates has been slight. Though somewhat lower concentration appears in the summer covercrop

plots than in the adjacent check, the differences are slight; those that do appear are greater in the lower than in the upper layers.

The plots on which *Melilotus indica* was grown as a winter covercrop have shown an anomalous behavior with respect to  $\text{NO}_3$  concentration.

TABLE 3  
NITRATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A,  
IN PARTS PER MILLION OF  $\text{NO}_3$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	June 3	350	310	650	910
	July 16	260	210	310	620
	September 30	520	330	560	980
Alfalfa	June 4	320	280	100	80
	July 17	750	280	150	100
	October 2	610	300	130	100
Summer covercrop	June 5	230	210	290	530
	July 18	410	230	140	400
	October 3	370	100	850	540
Clean-cultivated check	June 6	280	320	670	1,160
	July 21	330	310	440	630
	October 8	670	460	620	640
Winter covercrop of melilotus	June 9	230	80	40	110
	July 22	360	140	70	90
	October 10	590	130	100	210
Winter covercrop of rye	June 10	300	140	80	70
	July 23	450	160	60	90
	October 14	540	200	80	50
Clean-cultivated check	June 11	310	300	430	580
	July 24	340	290	480	570
	October 16	900	550	970	590

Although most plots show an increase in  $\text{NO}_3$  over the check, in the top soil, the reverse is true in the lower depths. In the late fall and early winter after seeding there is usually a decrease of  $\text{NO}_3$  in those plots as compared with the checks.

The plants were well nodulated and were plowed under while still succulent; but, despite this fact, the influence of melilotus, generally, has been to depress the  $\text{NO}_3$  concentration in the lower depths of the soil.

The effect of a winter covercrop on  $\text{NO}_3$  in the lower depths is carried still further in the case of rye. Although the top 2 feet often show an

increase over the check, the lower depths show a greater depression of the  $\text{NO}_3$  concentration. The concentration of  $\text{NO}_3$  in the 6-8 foot layer of the check often is 20 to 30 times that occurring at the same depth in

TABLE 4  
NITRATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B,  
IN PARTS PER MILLION OF  $\text{NO}_3$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	March 28	390	410	900	1,030
	May 14	420	410	560	570
	June 23	300	330	530	480
	August 23	510	.....	200	750
	December 6	690	410	650	670
Alfalfa	March 22	300	280	80	60
	May 15	630	410	90	100
	June 24	590	230	130	70
	August 28	980	340	310	130
	December 13	860	510	390	160
Summer covercrop	May 16	340	330	510	800
	June 25	390	270	280	640
	August 29	400	270	440	920
	December 15	.....	370	590	740
Clean-cultivated check	May 19	320	330	810	660
	June 25	290	390	760	710
	August 30	240	280	670	580
	December 17	.....	360	770	750
Winter covercrop of melilotus	May 20	450	190	350	370
	June 27	460	170	250	350
	September 2	490	240	160	250
	December 18	370	330	310	260
Winter covercrop of rye	May 21	510	290	460	240
	June 30	520	190	190	220
	September 3	400	170	140	230
	December 19	760	210	250	200
Clean-cultivated check	May 22	320	490	690	780
	July 1	350	320	540	700
	September 11	420	270	260	310
	December 22	330	590	630	540

the rye plot. These high ratios were not found in the data for 1930 given here but were present in some of the data for other years. This result can be interpreted in several ways: there may be direct absorption by roots of the covercrop; the carbohydrate materials of the plant may be leached throughout the soil column examined, providing a source of energy for organisms which remove  $\text{NO}_3$  from the solution to build

protoplasm; or the decomposition of rye may produce substances which inhibit the activity of nitrifying organisms. A somewhat similar circumstance has been reported by Batchelor,<sup>3</sup> who found that straw added to the surface soil of citrus orchards reduced the  $\text{NO}_3$  content of the top 4 feet of soil to a negligible amount. In that case the first possibility, that of removal of  $\text{NO}_3$  by the roots of a covercrop, is eliminated, and depression of  $\text{NO}_3$  must be ascribed to leached materials of one of the two kinds postulated above. This explanation will also account for the fact that the  $\text{NO}_3$  concentration of the lower depths in the summer cover and the melilotus plots is less than that of the checks.

The contrast between peaches and pears is difficult to explain with the existing data. The differences in the top 4 feet are in accord with expectations, but the reversal of this relationship in the lower 4 feet is not. It cannot be explained on the basis of root distribution, there being many roots in this region, as shown by their extraction of moisture and by their presence in samples. It cannot be explained by leaching from the surface soil. The concentration is greater in the lower depth and, too, is higher under peaches, where the  $\text{NO}_3$  concentration in the surface soil is lower than under pears. Apparently there may be nitrification at these depths at a rate greater than the rate of withdrawal, and the differential for peaches may excel that for pears. Further work on this point is necessary before definite conclusions can be drawn.

Certain observations that bear on interpretation of these phenomena should now be made. Because of the low annual rainfall (about 17 inches) and its distribution almost exclusively through the winter months, the soil is not leached by rain water below the depth of root penetration.

Irrigation is practiced in the summer, the water carrying an appreciable amount of salts. The concentrations in parts per million are as follows: totals solids at  $105^\circ\text{C}$ , approximately 500; Na, about 50; K, 1; Ca, 35; Mg, 60; Cl, 10;  $\text{HCO}_3$ , 400;  $\text{NO}_3$ , 4;  $\text{SO}_4$ , 25; and  $\text{SiO}_2$ , 40. This water is applied at a rate that does not always effect penetration to the full depth of sampling. In 1932, two irrigations at very short intervals were given in order to wet the entire 8-foot column. Moisture data indicate that during prolonged periods the soil below 6 feet, in several plots at least, has had no additions of water from the surface. This being the case, displacement of nitrates from higher levels into this region cannot have been a factor in causing the high concentration observed. The variations noted must result from biological factors.

<sup>3</sup> Personal correspondence.

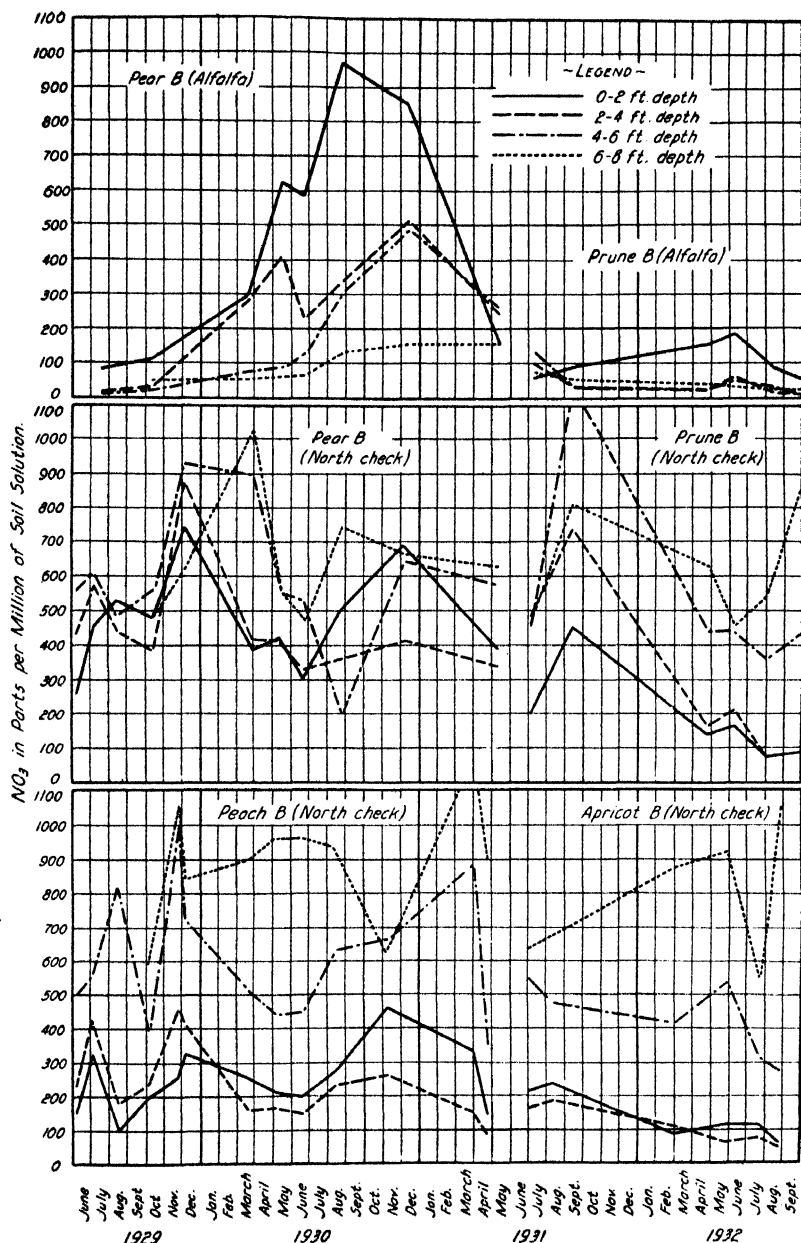


Fig. 2. Nitrate concentration of the soil solution from four depths in each of three plots, showing seasonal, species, and treatment differences.

On the other hand, the addition of water, whether by rain or by irrigation, must dilute the  $\text{NO}_3$ , at least temporarily. There must be some displacement of  $\text{NO}_3$  into lower layers, also, even though it does not always proceed to the limit of sampling. The amount of displacement under field conditions is a matter of conjecture. It no doubt varies with the number of root channels and fissures, as indicated by Slater and Byers<sup>(6)</sup>. Such changes of concentration as may be ascribed to these causes are less than the changes actually observed, and do not affect the generalizations made concerning seasonal trends and differences between plots. In certain cases aberrant results may be ascribed to these factors, but they are not of first importance.

The changes in  $\text{NO}_3$  concentration under apricots resemble those under peaches so closely as to permit these fruits to be discussed together. This fact is illustrated in figure 2. The general level of the curves is practically continuous for all layers except the surface. The surface 2 feet shows a lower concentration under apricots than under peaches, but this may be only a seasonal effect.

The prune plots yield  $\text{NO}_3$  concentrations more like those of pear plots than of the other stone fruits. The fact that prune trees are smaller and less vigorous than the other stone fruits considered may be the explanation. The prune plot trends are exemplified in figure 2, where the data are plotted as continuations of the pear curves. These figures also show the contrast between check and alfalfa plots.

As the figures indicate, the surface soil appears to be more regular in the sequence of changes than the lower depths. This phenomenon does not, apparently, result from sampling errors, for duplicate determinations have usually given very similar concentrations. The fact that each sample is a composite of 30 cores from the soil tube, taken in an area roughly  $20 \times 50$  feet, accounts for the small error in sampling. The data at hand do not explain all the deviations from smooth curves. Seemingly, however, these deviations do not occur often enough to invalidate the general conclusions.

The difference between the surface 2 feet and the second 2 feet might be tentatively explained as resulting from the higher organic content of the top soil and from the greater root distribution in the 2-4 foot layer. A more scattered root system might be postulated to account for the increased concentration below that depth; but, as indicated above, the data at hand are not deemed adequate for a satisfactory explanation of the facts observed.

## SULFATE

The data for sulfate concentration are presented in tables 5 to 8. The relationships pointed out in earlier papers for the top 4 feet hold for this region. In the lower layers, a curious circumstance appears: the inverse

TABLE 5  
SULFATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK A,  
IN PARTS PER MILLION OF  $\text{SO}_4$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	May 23.....	160	260	280	180
	July 2.....	130	200	240	140
	September 12.....	290	420	260	140
	December 23.....	280	330	330	170
Alfalfa.....	May 25.....	100	180	230	320
	July 7.....	110	140	180	230
	September 18.....	80	170	180	200
Summer covercrop.....	May 27.....	140	190	290	270
	July 8.....	120	150	220	260
	September 14.....	260	230	420	350
Clean-cultivated check.....	May 28.....	120	270	280	170
	July 9.....	100	270	290	200
	September 19.....	200	360	480	240
Winter covercrop of melilotus.....	May 29.....	190	310	270	160
	July 10.....	.....	310	300	140
	September 20.....	220	510	350	370
Winter covercrop of rye.....	May 30.....	170	260	260	160
	July 11.....	150	330	270	120
	September 25.....	180	240	360	190
Clean-cultivated check.....	May 31.....	150	280	310	150
	July 15.....	.....	340	270	140
	September 27.....	220	390	340	140

relationship noted between  $\text{SO}_4$  concentration and  $\text{NO}_3$  concentration in the surface soil does not hold. That is, even though the  $\text{NO}_3$  concentration is higher under peaches than under pears at these depths, the  $\text{SO}_4$  concentration is also higher. The same phenomenon is seen in the comparison of apricots with prunes, the apricots exhibiting a condition analogous to that of peaches, the prunes to that of pears.

Another consistent relationship noted is that the maximum concentration of  $\text{SO}_4$  occurs in the 4-6 foot layer. This condition obtains almost



without exception except in alfalfa, where the concentration tends to be nearly the same in the two lower layers.

As in earlier years, more irregularity is apparent in the seasonal curves of  $\text{SO}_4$  concentration than in those of  $\text{NO}_3$ .

TABLE 6  
SULFATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK B,  
IN PARTS PER MILLION OF  $\text{SO}_4$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	March 21.....	110	190	220	150
	May 5.....	200	210	230	200
	June 20.....	150	210	280	190
	August 12.....	160	240	300	220
	November 7.....	300	350	250	.....
Alfalfa.....	March 1.....	80	100	90	80
	May 6.....	90	100	90	70
	June 19.....	100	100	110	90
	August 13.....	100	120	140	110
	November 8.....	220	230	90	130
Summer covercrop.....	May 7.....	170	220	270	300
	June 18.....	160	210	280	270
	August 14.....	100	290	350	270
	November 14.....	240	350	360	290
Clean-cultivated check.....	May 8.....	150	190	280	230
	June 17.....	120	190	280	260
	August 15.....	210	200	270	210
	November 23.....	60	280	360	290
Winter covercrop of melilotus.....	May 9.....	150	290	210	140
	June 16.....	160	160	210	320
	August 16.....	180	290	270	210
	November 25.....	50	310	320	190
Winter covercrop of rye.....	May 12.....	140	220	220	190
	June 13.....	130	180	240	220
	August 21.....	170	260	340	200
	November 29.....	230	.....	.....	280
Clean-cultivated check.....	May 13.....	140	210	280	170
	June 12.....	100	220	270	230
	August 22.....	220	290	370	150
	December 2.....	180	220	320	210

There have been significant additions of sulfate in the irrigation water (see concentrations, page 560). The annual increment would be approximately 100 pounds per acre, or about 30 p.p.m. for the 8 feet calculated on an average water content. This figure is no more than a rough estimate to give the order of magnitude of the additions made. On the

basis of this estimate, enough sulfate has been added to give 250 to 300 p.p.m. of solution in the 8-foot column in the past ten years. That the solution has not been increasing notably in this period, certainly

TABLE 7  
SULFATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A,  
IN PARTS PER MILLION OF  $\text{SO}_4$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	June 3	160	130	160	140
	July 16	100	130	190	170
	September 30	140	200	200	180
Alfalfa	June 4	70	200	180	270
	July 17	90	110	160	150
	October 2	80	110	170	190
Summer covercrop	June 5	110	180	230	150
	July 18	50	170	120	170
	October 3	170	230	360	160
Clean-cultivated check	June 6	180	170	180	110
	July 21	130	200	180	120
	October 8	230	290	250	180
Winter covercrop of melilotus	June 9	90	250	160	120
	July 22	120	210	160	60
	October 10	140	300	230	160
Winter covercrop of rye	June 10	70	150	150	110
	July 23	140	190	180	140
	October 14	110	140	190	160
Clean-cultivated check	June 11	110	160	210	130
	July 24	150	180	200	120
	October 16	210	250	220	110

not in any such amount, may indicate a biological control of  $\text{SO}_4$  concentration within certain limits. Precipitation of  $\text{CaSO}_4$  seems unlikely because the concentration of Ca and  $\text{SO}_4$  are well below the solubility of this compound, and fluctuate from time to time. It would be expected that equilibrium with solid  $\text{CaSO}_4$  would give a nearly constant concentration. The annual cycle of changes cannot be interpreted on the basis of increments added by irrigation water, although these increments may be a factor in the irregularity noted.

SULFATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B,  
IN PARTS PER MILLION OF  $\text{SO}_4$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	March 28.....	150	140	170	140
	May 13.....	210	130	120	80
	June 23.....	100	140	130	90
	August 23.....	90	160	190	120
	December 6.....	210	170	180	110
Alfalfa	March 22.....	50	90	80	90
	May 14.....	80	100	90	90
	June 24.....	80	90	90	100
	August 28.....	110	90	120	130
	December 13.....	80	100	110	100
Summer covercrop	May 15.....	100	110	170	160
	June 25.....	160	160	160	160
	August 29.....	100	140	180	180
	December 15.....	150	190	200	190
Clean-cultivated check	May 19.....	120	120	150	100
	June 25.....	80	140	150	120
	August 30.....	160	170	120	80
	December 17.....	.....	160	170	100
Winter covercrop of melilotus	May 20.....	150	190	90	100
	June 27.....	100	140	150	110
	September 2.....	130	110	190	80
	December 18.....	.....	100	110	.....
Winter covercrop of rye	May 21.....	120	220	180	100
	June 30.....	130	200	190	110
	September 3.....	90	120	160	90
	December 19.....	170	.....	260	130
Clean-cultivated check	May 22.....	110	150	140	130
	July 1.....	50	140	150	110
	September 11.....	130	130	160	120
	December 22.....	100	200	190	110

## BICARBONATE

The  $\text{HCO}_3$  concentration also shows a tendency to fall off during the growing season in the surface 4 feet. This trend, as shown in tables 9 to 12, does not appear in some plots, and is of a low order in others. In

TABLE 9

BICARBONATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK A,  
IN PARTS PER MILLION OF  $\text{HCO}_3$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	May 23	60	70	110	140
	July 2	60	70	180	170
	September 12	40	.....	100	120
Alfalfa	May 26	80	100	210	220
	July 7	130	90	180	240
	September 18	110	100	180	250
Summer covercrop	May 27	90	80	160	200
	July 8	100	60	200	260
	September 14	.....	.....	130	250
Clean-cultivated check	May 28	70	70	160	200
	July 9	.....	70	140	.....
	September 19	60	50	120	220
Winter covercrop of melilotus	May 29	100	70	150	240
	July 10	.....	80	180	290
	September 20	130	70	110	130
Winter covercrop of rye	May 30	80	80	160	270
	July 11	110	80	200	320
	September 25	150	130	200	360
Clean-cultivated check	June 2	60	60	140	140
	July 12	.....	70	140	120
	September 27	170	50	140	150

the lower layers the falling off is likewise not entirely regular. The data obtained from the apricot and prune series in 1931 are still less consistent in the depths below 4 feet.

One very regular relationship, however, is the much higher concentration of  $\text{HCO}_3$  in the lower than in the upper layers, in all plots in all series in all years.

Another such relationship is the higher concentration in solutions from alfalfa plots. This difference is less than that between top and

lower soils, but is nearly as consistent throughout the period dealt with. Very few exceptions occur in the many samples compared. The winter-covercrop plots show a generally increased  $\text{HCO}_3$  concentration, com-

TABLE 10

BICARBONATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK B,  
IN PARTS PER MILLION OF  $\text{HCO}_3$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	March 21.....	190	60	140	140
	May 5.....	110	100	180	160
	June 20.....	60	70	130	130
	August 12.....	90	70	150	120
	November 7.....	60	50	.....	120
Alfalfa	March 1.....	280	150	200	320
	May 6.....	110	110	210	310
	June 19.....	100	110	170	270
	August 13.....	140	130	130	210
	November 9.....	70	70	110	170
Summer covercrop	May 7.....	120	100	210	310
	June 18.....	80	70	170	220
	August 14.....	60	30	100	110
	November 14.....	80	60	110	180
Clean-cultivated check	May 8.....	100	80	170	300
	June 17.....	80	60	140	190
	August 15.....	100	100	160	250
	November 23.....	40	20	50	60
Winter covercrop of melilotus	May 9.....	100	90	190	270
	June 16.....	130	90	180	180
	August 16.....	110	70	180	250
	November 25.....	50	50	90	100
Winter covercrop of rye	May 12.....	140	100	200	270
	June 13.....	150	130	180	230
	August 21.....	90	90	150	260
	November 29.....	150	60	110	150
Clean-cultivated check	May 13.....	70	70	150	130
	June 12.....	120	70	140	130
	August 22.....	80	60	120	140
	December 2.....	30	30	70	80

pared with the checks. Their values are sometimes greater than those for alfalfa, especially in the rye plots.

The amount of  $\text{HCO}_3$  added by the irrigation water is of considerable magnitude. Apparently, however, only biological processes in the soil can account for the observed concentration.

Burd and Martin's<sup>(1)</sup> hypothesis, that anions may be absorbed more rapidly than cations, and  $\text{HCO}_3$  excreted by the organism to preserve the electrical balance, seems the most acceptable one at the present time. If monovalent ions are more readily absorbed than divalent, then one

TABLE 11

BICARBONATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A,  
IN PARTS PER MILLION OF  $\text{HCO}_3$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	{ June 3.....	50	50	140	210
	{ July 16.....	50	50	180	270
	{ September 30.....	40	40	140	230
Alfalfa.....	{ June 4.....	60	70	200	220
	{ July 17.....	60	80	210	280
	{ October 2.....	50	60	180	170
Summer covercrop.....	{ June 5.....	70	50	200	230
	{ July 18.....	50	80	200	280
	{ October 3.....	50	40	130	190
Clean-cultivated check.....	{ June 6.....	40	30	140	120
	{ July 21.....	40	40	180	170
	{ October 8.....	70	30	130	140
Winter covercrop of melilotus.....	{ June 9.....	120	80	270	460
	{ July 22.....	60	40	220	300
	{ October 10.....	40	40	160	200
Winter covercrop of rye.....	{ June 10.....	150	90	250	380
	{ July 23.....	90	50	200	310
	{ October 14.....	40	40	190	300
Clean-cultivated check.....	{ June 11.....	80	90	170	240
	{ July 24.....	40	40	190	310
	{ October 16.....	90	40	140	260

might expect a greater absorption of cations in the surface soil, where K concentration is greatest (see below). In the deeper soil, the decreased K concentration and increased Ca and Mg concentration would tend to decrease cation absorption. The higher  $\text{NO}_3$  concentration might tend to encourage a relatively increased absorption of anions. The  $\text{HCO}_3$  concentration under these conditions would tend to be low in the surface layers and high in the lower ones, as is actually the case. Such a process would likewise tend to shift the pH toward the alkaline side in the lower layers; and this theory again fits the facts, the pH in the top 4 feet ranging about 7.6, while the 4-8 foot column is approximately 8.2.

TABLE 12  
BICARBONATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B,  
IN PARTS PER MILLION OF  $\text{HCO}_3$  OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	March 28	70	60	170	160
	May 14	50	60	180	190
	June 23	40	60	190	170
	August 23	50	80	170	210
	December 6	20	20	120	150
Alfalfa	March 22	180	80	250	260
	May 15	80	70	220	240
	June 24	70	60	210	260
	August 28	....	60	160	250
	December 13	20	20	100	240
Summer covercrop	May 16	70	70	210	320
	June 25	80	70	170	250
	August 29	80	50	130	170
	December 15	20	10	80	110
Clean-cultivated check	May 19	60	50	170	290
	June 26	40	40	150	160
	August 30	70	170	200	100
	December 17	....	20	90	220
Winter covercrop of melilotus	May 20	70	60	190	330
	June 27	60	40	180	350
	September 2	80	50	130	190
	December 18	20	30	110	.....
Winter covercrop of rye	May 21	60	50	170	310
	June 30	60	20	160	270
	September 3	50	40	170	340
	December 19	20	70	140	140
Clean-cultivated check	May 22	40	50	160	190
	July 1	30	30	140	130
	September 11	50	60	140	250
	December 22	10	20	100	120

There are no carbonates in this soil in the 8 feet used for these samples. As an alternative explanation, it has been suggested by Burd<sup>4</sup> that liberation of  $\text{CO}_2$  from the soil in consequence of slight changes in the buffer system might adequately account for the differences in  $\text{HCO}_3$  shown in these analyses.

<sup>4</sup> Personal correspondence.

## CHLORIDE

For the years 1931 and 1932, chloride determinations were made on all solutions. The data for these solutions (numbering over 800) are not presented, because their significance does not seem to warrant the space. Chloride, not being considered an important nutrient, was not included in the analysis of earlier solutions; it was included for 1931 and 1932 primarily to enable a closer balance sheet of cations and anions to be prepared. There is some similarity between Cl concentration and  $\text{SO}_4$  concentration. It is low in the surface, with a maximum in the 4-6 foot layer. It is higher under peaches than under pears, and higher under apricots than under prunes. It is low in alfalfa plots and intermediate in the winter-covercrop plots, as compared with the checks. The concentration in the 0-2 foot layer of pears averages about 60 parts per million; in the 2-4 foot layer, slightly more; in the 4-6 foot layer, 80 to 160 p.p.m.; and in the 6-8 foot layer, 60 to 120 p.p.m. In the peaches the averages range from 40 to 70 p.p.m. in the surface layer; 70 to 110 in the 2-4 foot layer; 120 to 300 in the 4-6 foot layer; and 150 to 260 in the 6-8 foot layer. The apricot plots give somewhat higher results than the peach; the prune little more than the pear. These results vary considerably in the two years, the 1931 levels being higher than those of 1932.

## PHOSPHATE

Only one point brought out by the new data adds to those illustrated by the previous figures as respects phosphate content. The  $\text{PO}_4$  concentration is greatest in the surface soil (about 0.3 p.p.m.), decreases to a minimum in the 4-6 foot layer, and rises slightly in the 6-8 foot depth. The level is low in all cases, with an average of less than 0.1 p.p.m. of  $\text{PO}_4$  in the 4-6 foot zone. There seems to be an equilibrium condition without seasonal change. In spite of this low level, the trees show no indication whatever of phosphorous deficiency. Growth has been vigorous. The constant concentration, even though low, seems to supply an adequate total amount. As others have pointed out, however, these values are averages; and local zones at the interface between soil particle and absorbing surface of the root may be entirely different in magnitude.



## CALCIUM

The results of the analyses for calcium appear in tables 13 to 16. These data have confirmed those presented before on the Ca concentration in the upper 4 feet. The general relationships for Ca concentration agree

TABLE 13

CALCIUM CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK A,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	{ June 23.....	60	83	196	199
	{ July 2.....	55	55	107	118
	{ September 12.....	103	151	.....	173
	{ December 23.....	115	100	193	140
Alfalfa.....	{ May 26.....	83	75	70	100
	{ July 7.....	97	50	60	72
	{ September 18.....	50	73	60	60
Summer covercrop.....	{ May 27.....	50	52	89	145
	{ July 8.....	68	45	80	135
	{ September 14.....	78	55	135	314
Clean-cultivated check.....	{ May 28.....	41	62	132	143
	{ July 9.....	53	56	134	269
	{ September 19.....	73	79	223	150
Winter covercrop of melilotus.....	{ May 29.....	61	74	186	142
	{ July 10.....	.....	76	135	131
	{ September 20.....	85	106	152	231
Winter covercrop of rye.....	{ May 30.....	60	72	103	65
	{ July 11.....	94	91	100	59
	{ September 25.....	88	82	128	78
Clean-cultivated check.....	{ June 2.....	59	70	162	203
	{ July 15.....	.....	78	150	235
	{ September 27.....	83	112	205	220

nicely with those recorded for  $\text{NO}_3$  above. There is, however, less contrast between surface and deeper layers than in the case of  $\text{NO}_3$ . In some of the pears, in fact, the deeper layers are actually lower in the Ca ion, notably in some of the rye-plot samples. The high nitrate content of the deeper layers under peaches is reflected in the high Ca content of the same regions. The apricot and prune plots also show the contrasts indicated in the discussion of  $\text{NO}_3$  above.

TABLE 14

CALCIUM CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK B,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	{ March 21.....	64	56	108	170
	{ May 5.....	67	60	110	196
	{ June 20.....	52	61	123	280
	{ August 12.....	57	80	141	180
	{ November 7.....	110	111	143	120
Alfalfa.....	{ March 1.....	67	43	40	53
	{ May 6.....	80	46	40	45
	{ June 19.....	65	48	43	48
	{ August 13.....	66	46	44	51
	{ November 8.....	120	69	30	58
Summer covercrop.....	{ May 7.....	53	71	137	180
	{ June 18.....	62	57	96	235
	{ August 14.....	42	84	153	246
	{ November 14.....	98	94	197	208
Clean-cultivated check.....	{ May 8.....	50	65	149	143
	{ June 17.....	46	47	131	184
	{ August 15.....	78	59	126	128
	{ November 23.....	33	71	157	238
Winter covercrop of melilotus.....	{ May 9.....	50	58	111	113
	{ June 16.....	60	47	77	114
	{ August 16.....	64	73	93	97
	{ November 25.....	30	81	100	99
Winter covercrop of rye.....	{ May 12.....	52	70	85	70
	{ June 13.....	57	57	81	79
	{ August 21.....	75	75	131	87
	{ November 29.....	88	88	130	105
Clean-cultivated check.....	{ May 13.....	56	76	132	179
	{ June 12.....	42	54	103	202
	{ August 22.....	90	87	131	184
	{ December 2.....	90	60	120	115

In a considerable number of samples, the Ca content has its maximum in the 4-6 foot layer, the 6-8 foot zone showing some decrease. This is a point of divergence from the behavior of nitrate.

A notable reduction of Ca appears in the lower layers of alfalfa and the rye plots and to a less extent in the melilotus plots.

TABLE 15  
CALCIUM CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	{ June 3.....	68	60	106	99
	{ July 16.....	50	50	79	81
	{ September 30.....	91	90	122	142
Alfalfa.....	{ June 4.....	42	81	71	51
	{ July 17.....	90	51	57	47
	{ October 2.....	80	56	58	47
Summer covercrop	{ June 5.....	50	62	89	70
	{ July 18.....	57	62	62	84
	{ October 3.....	76	63	111	41
Clean-cultivated check	{ June 6.....	69	70	119	113
	{ July 19.....	61	75	101	84
	{ October 8.....	127	113	156	100
Winter covercrop of melilotus	{ June 9.....	58	58	63	55
	{ July 22.....	70	60	65	42
	{ October 10.....	96	77	80	52
Winter covercrop of rye	{ June 10.....	65	51	61	43
	{ July 23.....	80	58	60	44
	{ October 14.....	85	57	64	43
Clean-cultivated check	{ June 11.....	60	75	88	62
	{ July 24.....	67	71	90	53
	{ October 16.....	159	133	162	66

TABLE 16  
CALCIUM CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	March 28.....	76	82	146	132
	May 14.....	94	78	107	75
	June 23.....	56	70	95	68
	August 23.....	73	76	114	98
	December 6.....	124	93	111	89
Alfalfa.....	March 22.....	56	52	51	43
	May 15.....	97	69	52	48
	June 24.....	73	47	54	48
	August 28.....	124	54	74	58
	December 13.....	105	72	67	42
Summer covercrop.....	May 16.....	59	56	93	110
	June 25.....	75	60	73	96
	August 29.....	47	57	85	109
	December 15.....	105	68	96	100
Clean-cultivated check.....	May 19.....	56	59	118	75
	June 26.....	54	73	123	82
	August 30.....	53	72	70	82
	December 17.....	....	73	110	77
Winter covercrop of melilotus.....	May 20.....	93	55	71	51
	June 27.....	83	53	66	58
	September 2.....	85	56	62	51
	December 18.....	60	63	67	....
Winter covercrop of rye.....	May 21.....	96	78	93	45
	June 30.....	93	69	66	45
	September 3.....	68	55	62	50
	December 19.....	112	73	85	37
Clean-cultivated check.....	May 2.....	58	78	110	104
	July 1.....	62	62	103	84
	September 11.....	87	62	76	68
	December 22.....	57	101	109	56

## MAGNESIUM

The magnesium content (given in tables 17-20) follows that of Ca with extraordinary fidelity in the top 4 feet. Though it is generally somewhat lower in the surface 2 feet than is Ca, the divergence is not

TABLE 17

MAGNESIUM CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK A,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	{ May 23.....	53	95	286	346
	{ July 2.....	48	32	162	216
	{ September 12.....	88	155	212	265
	{ December 3.....	103	102	266	265
Alfalfa.....	{ May 26.....	72	61	91	149
	{ July 7.....	87	45	72	95
	{ September 18.....	41	61	66	82
Summer covercrop.....	{ May 27.....	45	56	108	249
	{ July 8.....	63	41	95	235
	{ September 14.....	80	67	147	261
Clean-cultivated check.....	{ May 28.....	37	75	179	343
	{ July 9.....	47	64	179	353
	{ September 19.....	79	96	222	295
Winter covercrop of melilotus.....	{ May 29.....	59	75	233	303
	{ July 10.....	.....	71	196	291
	{ September 20.....	78	84	125	144
Winter covercrop of rye.....	{ May 30.....	53	72	128	141
	{ July 11.....	83	86	117	146
	{ September 25.....	81	82	137	171
Clean-cultivated check.....	{ June 2.....	57	74	189	315
	{ July 15.....	.....	88	168	204
	{ September 27.....	79	117	245	297

great. In the 6-8 foot zone, however, appears a marked divergence, Mg being much higher in many plots. In the alfalfa, rye, and melilotus plots, both Ca and Mg are reduced to a low level in the region below 4 feet; but in the check plots and in the summer covercrop plots the relationship indicated is rather consistent for all fruits studied. Possibly Mg is more easily leached through a soil than is Ca, and has accumulated in the lower depths as a result of such leaching over the period of soil

TABLE 18  
MAGNESIUM CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK B,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	March 21	52	66	119	155
	May 5	62	69	118	198
	June 20	42	53	133	143
	August 12	49	83	161	188
	November 7	103	110	160	122
Alfalfa	March 1	60	41	42	57
	May 6	71	46	46	47
	June 19	60	42	45	51
	August 13	57	44	48	55
	November 8	118	77	29	66
Summer covercrop	May 7	47	78	166	247
	June 18	59	56	107	196
	August 14	38	91	170	297
	November 14	90	101	237	310
Clean-cultivated check	May 8	40	66	165	227
	June 17	40	50	137	208
	August 15	74	62	136	234
	November 23	29	84	232	316
Winter covercrop of melilotus	May 9	46	65	121	154
	June 16	44	42	83	132
	August 16	63	72	97	147
	November 25	18	91	105	147
Winter covercrop of rye	May 12	47	70	88	87
	June 13	45	55	72	89
	August 21	68	73	131	107
	November 29	84	94	133	129
Clean-cultivated check	May 13	46	76	134	200
	June 12	37	48	110	191
	August 22	80	90	140	198
	December 2	84	57	126	134

formation. No points noted in these data would indicate that the concentration of Ca and Mg at any depth at any time is not primarily a function of biological activity and, in particular, of organisms affecting the nitrogen cycle.

TABLE 19

MAGNESIUM CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check.....	{ June 3.....	67	68	151	210
	{ July 16.....	46	55	114	203
	{ September 30.....	87	94	165	232
Alfalfa.....	{ June 4.....	42	97	108	121
	{ July 17.....	97	55	80	104
	{ October 2.....	83	59	79	107
Summer covercrop.....	{ June 5.....	47	64	119	146
	{ July 18.....	51	64	66	136
	{ October 3.....	67	61	212	96
Clean-cultivated check.....	{ June 6.....	99	82	146	217
	{ July 21.....	48	80	122	146
	{ October 8.....	122	126	162	153
Winter covercrop of melilotus.....	{ June 9.....	59	69	89	96
	{ July 22.....	71	71	89	74
	{ October 10.....	97	92	97	91
Winter covercrop of rye.....	{ June 10.....	59	47	64	91
	{ July 23.....	70	61	82	91
	{ October 14.....	83	58	85	88
Clean-cultivated check.....	{ June 11.....	46	59	116	144
	{ July 24.....	55	65	128	144
	{ October 16.....	136	121	217	142

TABLE 20  
MAGNESIUM CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B,  
IN PARTS PER MILLION OF DISPLACED SOLUTION

Treatment	Date	Depth			
		0-2 feet	2-4 feet	4-6 feet	6-8 feet
Clean-cultivated check	March 28.....	60	73	153	158
	May 14.....	79	77	120	106
	June 23.....	46	65	114	99
	August 23.....	54	58	141	125
	December 6.....	106	82	127	130
Alfalfa	March 22.....	52	59	56	55
	May 15.....	97	80	58	61
	June 24.....	74	53	64	70
	August 28.....	119	49	92	79
	December 13.....	105	56	81	58
Summer covercrop	May 16.....	58	67	122	177
	June 25.....	70	69	92	160
	August 29.....	31	60	107	186
	December 15.....	90	86	120	145
Clean-cultivated check	May 19.....	53	61	147	148
	June 26.....	46	78	141	169
	August 30.....	46	76	154	87
	December 17.....	...	80	135	166
Winter covercrop of melilotus	May 20.....	90	62	94	104
	June 27.....	78	55	84	108
	September 2.....	75	59	61	79
	December 18.....	53	66	81	.....
Winter covercrop of rye	May 21.....	90	89	117	93
	June 30.....	91	71	87	91
	September 3.....	47	41	34	66
	December 19.....	113	82	110	78
Clean-cultivated check	May 22.....	53	92	129	144
	July 1.....	57	72	19	77
	September 11.....	78	66	83	93
	December 22.....	54	113	135	112



## POTASSIUM

The data concerning K concentration of the solutions studied bring out nothing new; they are therefore omitted. The K concentration decreases with depth. It is constant throughout the year, with minor fluctuations, indicating an equilibrium with the solid phase. Differences between fruits or between treatments are too slight and irregular to be given any importance. The concentration is rather low, averaging about 6 p.p.m. in the top 2 feet, less than 2 p.p.m. at 2–4 feet, and less than 1 p.p.m. below 4 feet; but it seems entirely adequate for normal growth of the trees. The point noted in the discussion of phosphate—that these are average values which may not represent the concentration at the absorbing surface—should be noted in this connection also.

## HYDROGEN ION CONCENTRATION

The pH of the displaced solutions has seemed not to vary enough to be significant. Of course, the changes effected by sampling, packing, and displacing might, by releasing  $\text{CO}_2$ , shift the pH slightly. Any shift from this cause is probably small, however, the solutions being alkaline. Perhaps the approximations reached by our methods are not accurate enough to justify the conclusion that changes in pH are of little importance. All the solutions are slightly alkaline. The surface soil generally has a pH of about 7.4 to 7.6. The alkalinity increases with depth to a pH of about 8.2 at 6–8 feet. As stated above, the hypothesis used to account for the  $\text{HCO}_3$  changes fits the facts of H ion concentration. In addition, organic matter decomposing in the upper soil might supply acids which would tend to give a more acid condition in that region.

## GROWTH AND FRUITING

The circumference of the trunk of the tree has been taken as a convenient measure of growth. The complete records are not presented; but table 21 gives the present circumference, representing growth for 11 years in the case of block A, and 10 years in that of block B. These figures show that in the first eight years of differential covercrop treatment, no important differences have developed in size of trees. Nor, apparently, is there any indication that the rate of growth of any group of trees is being affected.

The time of leaf fall in the autumn of 1932 has not been affected by any treatment. Differences that appeared in the cherry and apricot

TABLE 21  
TRUNK CIRCUMFERENCES OF INDIVIDUAL TREES ON COVERCROP PLOTS, IN CENTIMETERS; NOVEMBER, 1932

Plot	Pear, A†		Prune, A		Apricot, A		Peach, A		Pear, B		Prune, B		Apricot, B		Peach, B	
	Row 2	Row 3	Row 4	Row 5	Row 12	Row 13	Row 14	Row 15	Row 1	Row 2	Row 3	Row 4	Row 11	Row 12	Row 13	Row 14
Guard row.....	58.8	65.5	24.9	46.2	79.8	81.8	82.4	82.5	82.6	37.1	51.8	21.3	94.3	82.0	84.7	91.3
Cheek.....	68.0	51.2	70.2	58.1	89.8	83.9	72.5	69.7	56.6	54.3	66.5	59.8	58.3	108.5	75.5	91.8
	78.0	59.9	64.7	63.1	75.2	80.4	82.8	90.2	57.6	51.4	43.3	57.6	85.6	85.6	63.2	96.5
	65.9	66.2	60.7	65.4	93.0	66.6	76.2	81.0	64.6	66.5	50.1	45.4	89.6	55.5	94.0	95.4
Alfalfa.....	60.4	57.7	54.8	51.4	out	90.1	80.6	99.7	52.5	4.9*	64.0	62.7	out	80.4	90.8	94.5
	58.7	42.2	59.0	67.2	84.8	90.5	90.1	91.2	54.1	43.6	59.0	56.3	75.5	84.5	94.7	83.4
	64.7	47.8	38.2	54.4	91.5	83.2	94.1	70.6	64.2	66.9	47.5	38.4	81.0	76.5	90.3	93.2
Summer covercrop.....	49.5	60.9	57.3	58.1	78.3	77.8	87.4	80.4	56.2	43.4	60.5	63.8	81.9	72.3	97.3	88.7
	55.2	50.8	65.2	65.2	86.7	82.2	104.0	96.7	48.1	54.1	62.1	68.8	68.7	75.2	91.6	94.6
	60.1	76.1	69.0	55.7	75.2	93.9	92.2	89.2	59.0	64.3	51.0	49.6	63.1	60.0	100.7	94.8
Cheek.....	79.6	69.3	40.5	63.6	70.4	82.7	69.5	90.4	54.5	60.4	74.3	66.7	79.4	67.5	82.5	91.2
	60.3	65.2	56.0	51.2	92.5	88.0	88.3	85.5	61.4	51.1	65.1	67.4	83.7	73.5	97.7	93.1
	58.0	70.7	35.1	67.4	84.1	83.0	70.2	82.6	62.7	67.3	56.9	46.1	87.9	83.6	98.5	92.5
Melilotus.....	44.1	63.2	51.8	69.9	88.2	80.8	80.3	96.4	53.9	22.9*	65.3	64.1	84.4	65.7	98.8	78.4
	52.9	58.5	63.2	67.3	92.5	84.5	80.8	94.7	57.8	50.9	61.4	64.5	82.8	73.4	103.6	97.3
	54.9	72.0	27.0	31.8	87.6	79.2	88.8	88.7	63.6	60.2	51.0	59.8	92.6	84.8	84.9	104.1
Rye.....	50.5	58.0	61.8	77.3	93.3	84.4	68.2	94.0	52.4	52.7	58.4	60.1	85.5	75.2	88.0	92.5
	60.8	66.3	58.2	62.6	84.4	83.7	93.0	89.0	47.5	54.0	77.6	69.4	81.0	93.0	71.0	90.6
	57.5	62.0	55.5	65.9	84.9	83.4	72.9	100.5	72.5	60.3	49.5	51.5	84.2	74.4	83.1	93.9
Cheek.....	63.2	57.6	65.1	56.5	85.5	76.2	83.5	86.4	54.4	5.0*	51.8	60.6	79.7	55.0	94.4	81.9
	65.2	64.6	63.0	46.7	90.7	83.3	84.5	89.0	84.0	40.7	68.1	58.5	73.3	75.6	81.8	80.9
	50.9	67.0	66.1	56.8	78.1	80.8	80.8	89.5	52.6	54.4	62.9	56.4	91.7	71.0	88.9	96.4

† Block A, 1922 planting; block B, 1923 planting.

\* Sprouts from trunk killed by blight.

series in 1930 seem to have been associated with moisture conditions rather than with nutrition. So far, therefore, one must conclude that treatments which profoundly modify the soil solution have not affected the growth of the trees. It remains to be seen how long a differential  $\text{NO}_3$  concentration can be maintained in the soil solution without affecting the growth of the tree.

TABLE 22

TOTAL KILOGRAMS OF FRUIT BORNE BY TREES IN COVERCROP EXPERIMENT

Plot	Pear, A (1932)	Prune, A (1930-32)		Apri- cot, A (1926- 32)	Peach, A (1926- 32)	Pear, B* (1932)	Prune, B (1930-32)		Apri- cot, B (1926- 32)	Peach, B (1926- 32)
		French (Agen)	Robe de Ser- geant				French (Agen)	Robe de Ser- geant		
Check.....	355	1,098	747	2,265	4,445	177	572	575	1,141	1,210
Alfalfa.....	382	1,009	440	1,943	4,017	218	649	391	936†	901
Summer cover- crop.....	350	1,218	683	2,138	3,830	236	685	703	868	1,581
Check.....	586	839	700	2,101	4,488	373	797	677	1,714	975
Melilotus.....	345	1,214	131	2,159	6,484	205	504	690	1,481	1,124
Rye.....	568	1,183	811	2,012	4,388	327	785	713	1,730	1,790
Check.....	377	688	333	2,017	4,362	218	494	611	1,354	2,690

\* Four trees of Bartlett per plot, the other two being Hardy, which have not as yet produced fruit.

† Five trees.

Fruit production records add little to the interpretation of the data at present. A summary giving the production per plot to date appears in table 22; it shows that the yields of some fruit are much more uniform than those of others. No treatment has resulted in consistently high yields. The trees commonly believed to use most  $\text{NO}_3$  seem not to have had their yields depressed more than those needing relatively little.

It may be stated in a sentence that after eight years' treatment no certain differences have developed in either growth or fruiting.

## SUMMARY

The data obtained from analyses of soil solutions displaced from 0-2, 2-4, 4-6, and 6-8 foot samples in peach, pear, apricot, and prune plots given differential covercrop treatments have shown that:

The average of the 0-2 and 2-4 foot samples confirms previously reported results.

The  $\text{NO}_3$  concentration in the 4-6 and 6-8 foot depths under peaches and apricots is higher than that under pears and prunes, in contrast to the opposite situation in the surface of 4 feet.

The  $\text{NO}_3$  concentration in the 4-6 and 6-8 foot samples is greatly reduced under alfalfa and winter covercrops as compared with clean-cultivated check plots.

Plowing under alfalfa increased the  $\text{NO}_3$  concentration strikingly in the surface 4 feet, but had little effect below that depth. Reseeding alfalfa caused a reduction of  $\text{NO}_3$  to about the former level.

The  $\text{SO}_4$  concentration under peaches and apricots is higher in the 4-6 and 6-8 foot samples than that under pears and prunes. The maximum  $\text{SO}_4$  concentration is usually in the 4-6 foot layer.

In spite of additions of  $\text{SO}_4$  by irrigation water, there has been little change in its concentration in the soil solution over the period studied.

The  $\text{HCO}_3$  concentration is higher in the 4-6 and 6-8 foot samples than in the 0-2 and 2-4 foot samples.

The  $\text{HCO}_3$  concentration is higher in the alfalfa and winter covercrop plots than in the checks.

The chloride concentration is higher in the lower than the upper layers, with a maximum at 4-6 feet.

The chloride concentration is higher under peaches and apricots than under pears and prunes, and lower under alfalfa than under clean cultivation.

The  $\text{PO}_4$  concentration is higher in surface than in deeper samples, with a minimum at 4-6 feet. There are no other significant differences, seasonal or from plot to plot.

The calcium concentration varies in the same manner as that of  $\text{NO}_3$ .

The magnesium concentration parallels that of calcium except that it is lower in the 0-2 foot and higher in the 6-8 foot samples than that of calcium.

The potassium concentration decreases with depth, but otherwise does not vary significantly.

## ACKNOWLEDGMENTS

Acknowledgment is made to O. C. Compton and C. O. Hesse, who secured the soil samples and aided in the laboratory; and to E. A. Wilkins, who made the potassium determinations referred to in this paper. The Division of Chemistry has supplied the analysis of the irrigation water.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

SEPTEMBER, 1933

No. 15

## WATERMELON BREEDING<sup>1</sup>

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### INTRODUCTION

Although the watermelon, *Citrullus vulgaris* Schrad., has been cultivated in America since 1629<sup>(9)</sup> and in Africa for over 4,000 years,<sup>(7)</sup> relatively little attention has been given to the effects of inbreeding, to environmental factors affecting fruit setting, or to measured varietal improvement through modern breeding methods. The effects of inbreeding assume economic importance because many watermelon varieties, normally subject to extensive cross-pollination, are apparently heterozygous as to many characters, particularly those affecting plant vigor; size, shape, and color of fruit; color and texture of flesh; sugar content; and certain seed characters.

Doubtless the most important improvement needed in watermelons is the development of strains resistant to the wilt disease, caused by *Fusarium niveum* E. F. S. Wilt is now a factor limiting production in the Sacramento, San Fernando, and San Joaquin valleys of California and in many other states. As the fungus is well established in the southern states, growers have some difficulty in locating disease-free soil. A single crop of watermelons often contaminates the soil to the extent that all subsequent crops may be seriously infected. Eventually, therefore, all the watermelon districts, each demanding a particular type of fruit, will probably need wilt-resistant strains.

As inbreeding must continue for several generations in order to establish homozygous wilt-resistant strains, workers evidently must (a) measure the effect of such inbreeding, (b) establish the mode of inheritance, (c) develop pollination technique, and (d) determine the occurrence of self-sterility. In the light of these needs, the work reported

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herein was initiated in 1929 and briefly reported in 1930.<sup>(14)</sup> Previous experience with the wilt disease in Iowa, already described,<sup>(15)</sup> emphasized the necessity of these investigations. The studies herein reported were conducted mainly with the Klondike variety.

## HISTORICAL

The effects of inbreeding have been more thoroughly investigated in the Cucurbitaceae than in any other family of vegetable crops. The earlier workers, Drude,<sup>(4)</sup> Lotsy,<sup>(10)</sup> and Sinnot and Durham,<sup>(20, 21)</sup> dealing with certain varieties of *Cucurbita pepo* noted the extreme difficulty of effecting self-fertilization and agreed that sterility and loss of vigor combined to hinder the establishment of pure lines. Bushnell,<sup>(2)</sup> however, working with Hubbard squash (*C. maxima*) at the Minnesota station, concluded that inbreeding tended to isolate strains of high quality and marked uniformity, yielding only slightly less than the commercial check lots, and that  $F_1$  hybrids between the inbred lines showed only slightly more vigor than the inbred lines, or about the same amount as the commercial check lots. Rosa,<sup>(18)</sup> working with *Cucumis melo* in California, showed that the second generation of inbred lines of the Salmon Tint cantaloupe might yield more or less than the parent variety; that lines differing in fruit shape from the parent variety could be isolated; and that there were slight differences in yield of seed, average weight per seed, and the development of fruit grooves and ribs. The report of Cummings and Jenkins<sup>(3)</sup> from the Vermont station indicated that in *Cucurbita maxima* continuous self-pollination did not influence seed viability nor induce degeneration of the species. Haber's<sup>(6)</sup> experience with *Cucurbita pepo* at the Iowa station showed the possibility of inbreeding the Table Queen (Des Moines) pumpkin and, at the same time, isolating strains that produce fruits of uniform size, shape, and quality. With the exception of preliminary studies made by the writer<sup>(14)</sup> and a brief report by Rosa,<sup>(16)</sup> breeding work with the watermelon has been limited to the development of resistant strains by Orton<sup>(11, 12, 13)</sup> and by Porter and Melhus.<sup>(15)</sup> Orton, although not attempting to cross pure lines, did report that extremely vigorous  $F_1$  hybrids resulted from crosses of the edible variety Eden with the nonedible "citron." By continued selection within hybrid material, he isolated and named the wilt-resistant variety Conquerer. In the writer's experience at the Iowa station, neither self-sterility nor apparent loss of vigor was encountered during three generations of inbreeding in the Kleckley Sweet watermelon.

Although, in general, neither consistent loss of vigor nor appreciable decrease in yield has appeared during inbreeding in the family Cucurbitaceae, just the reverse is true of *Zea mays*, likewise chiefly cross-fertilized. As shown by the work of East,<sup>(5)</sup> Shull,<sup>(22)</sup> and others on maize, marked loss of vigor results from inbreeding the progeny of individual plants for three or four generations. From the fifth to the seventh generations, apparently, the inbred lines approach homozygosity for the factors governing yield.

## FLOWERING HABIT AND FLORAL STRUCTURE

Most watermelon varieties produce staminate and pistillate flowers separately in the axils of the leaves. In some varieties—Angeleno, Chilean, Baby Delight (Hungarian Honey), and Winter Queen—hermaphroditic flowers are often, but not always, borne in place of pistillate. They produce an abundance of pollen, apparently indistinguishable from that produced by staminate flowers on the same plant. Rosa<sup>(16)</sup> has reported, and the writer has observed, that pistillate flowers are not fertilized if bagged before anthesis, a fact indicating the complete absence of parthenocarpy in several varieties of the species.

The ratio of pistillate or hermaphroditic to staminate flowers is not constant. Although actual counts have indicated a ratio of approximately 1 to 7 in the most important commercial varieties, female flowers are sometimes found at every third, fourth, ninth, or tenth node. They have, furthermore, been observed at adjacent nodes as well as in pairs at a single node. Though hermaphroditism might be considered desirable, as serving to increase the probability of self-pollination, no one, apparently, has attempted to introduce this characteristic into Klondike or other important commercial varieties. Obviously, not all the pistillate flowers set fruit; indeed, the average number of fruits per plant in Klondike (commercial lots) is probably less than 6 under field conditions, while many plants form 40 or more pistillate flowers during the blooming period.

Though the Klondike flower type is adapted to complete cross fertilization, the amount of natural crossing in the field has never been measured. Apparently, however, the first pistillate flowers to open, on a given plant, are more likely to be self-pollinated than those that open after the vines begin to intermingle. Pollination seems to be almost entirely the work of insects.

The number of flowers that open daily on a given plant is exceedingly variable, probably depending upon the age and vigor of the plant and



upon environmental factors during the preceding day and night. Under normal growing conditions, open female flowers are usually found 12 to 18 inches from the outer extremity of the runner. If there is insuffi-



Fig. 1.—Watermelon pollination, showing three stages in the life history of the pistillate flower. Left, open pistillate flower with a wilted corolla and withered stigma 24 hours after anthesis, showing open staminate flower at a *younger* node. Middle, pistillate flower two hours after anthesis, showing dehiscent staminate flower at an *older* node. Right, pistillate flower about 24 hours before anthesis, showing staminate flower at an *older* node.

cient soil moisture, abnormally high temperature, or disease infection, flowers may open within 2 inches of this outer extremity. In such cases, however, fertilization has not been observed to follow pollination.

Under environmental and cultural conditions in the Sacramento and San Joaquin valleys of California during June, July, and August, both pistillate and staminate flowers usually open between 6 and 8 a.m. The time apparently depends upon the air temperature during the preceding night. If the night is unusually warm ( $60^{\circ}$  to  $70^{\circ}$  F), many flowers are fully open by 6 a.m.; if cool, the time of flower opening is delayed. Pollen is often shed one to three hours before anthesis; and dehiscence often continues until late afternoon, when the corolla closes and the anthers begin to wither. These investigations have not definitely determined the length of time the pollen remains viable, nor the relation of temperature to rate of pollen germination and pollen tube growth. The stigmatic surface appears receptive at anthesis and generally remains sticky until the corolla closes.

Although anthesis of pistillate and staminate flowers is usually simultaneous, in the former case it is almost invariably found at the younger node on a given runner (fig. 1) and is usually accompanied by one to three open staminate flowers at nodes immediately preceding. In hermaphroditic flowers, anthesis seems to be considerably retarded. Within their closed corolla, pollen may be shed and the stigma may appear receptive 24 hours before anthesis. At this time, self-fertilization may be effected if the corolla is opened artificially and the pollen applied to the stigmatic surface. In hermaphroditic flowers at anthesis, the ovaries and stigmas are relatively larger than in purely pistillate flowers of the same variety.

### POLLINATION TECHNIQUE

The technique of artificial self-pollination is essentially the same for all varieties of *Citrullus vulgaris* except the hermaphroditic; in the latter case, staminate flowers need not be bagged before anthesis, as the hermaphroditic ones produce sufficient pollen. The following description applies to varieties producing purely staminate and pistillate flowers. Within 24 hours (or less) before anthesis, pistillate and staminate flowers are selected and are covered with a small muslin bag having a draw string (figs. 2, 3, and 4). The former are always younger than the latter and are located at nodes farther from the crown of the plant. As soon as possible after anthesis, these cloth bags are removed, the staminate flower is pinched off, and pollen is applied to the stigmatic surface. A one-pound manila paper bag is then slit for about three inches down each side, folded tightly over the pollinated flower, and fastened with a clip (fig. 5). After two days, this paper bag is removed. In making crosses involving hermaphroditic flowers, one must emasculate before

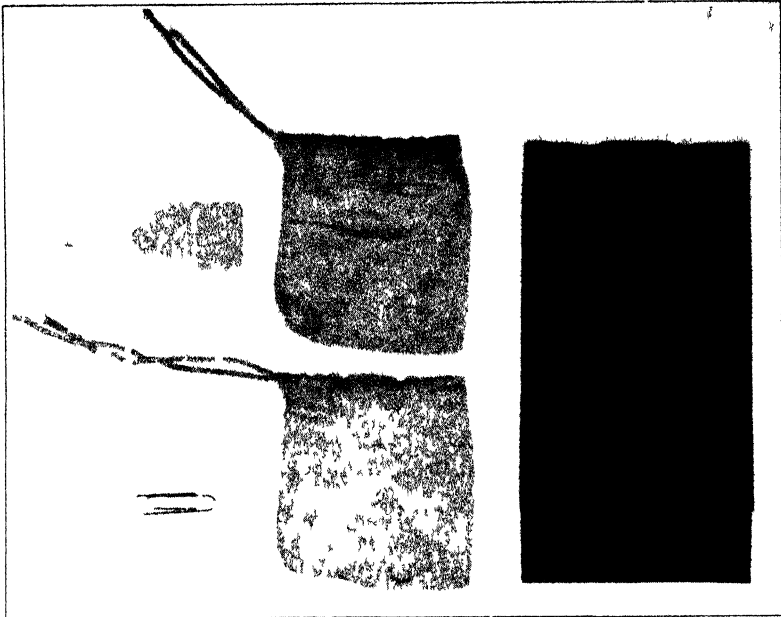


Fig. 2.—Equipment used in watermelon pollination. Two cloth bags for covering flower buds, one pound manila paper bag for screening pistillate flowers after pollination, paper chip for holding paper bag in place to prevent insect visitation; and label for recording pollination data as explained in text

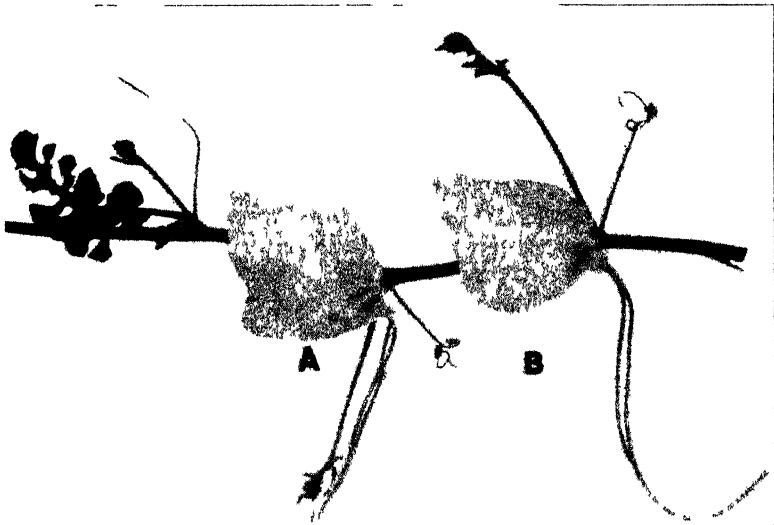


Fig. 3.—Watermelon pollination, showing cloth bags in place over pistillate flower *A* and staminate flower *B*. These bags are placed over the flower buds during the afternoon or evening immediately preceding anthesis. They are rarely entered by insects.

dehiscence as well as before anthesis. Experience has shown that if emasculation is delayed until pollen has dehiscid, contamination may result.

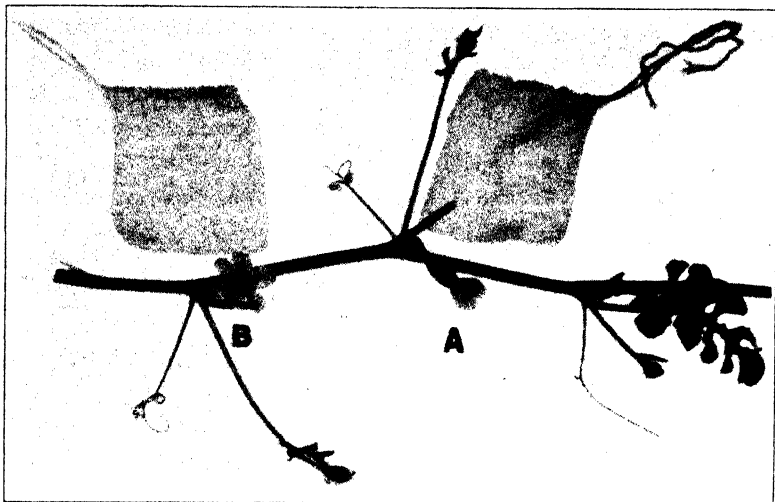


Fig. 4.—Watermelon pollination. Cloth bags removed during the morning, showing receptive pistillate flower *A*, and dehiscing staminate flower *B*. Note the relative position of these two flowers, with the staminate flower at an older node than the pistillate.

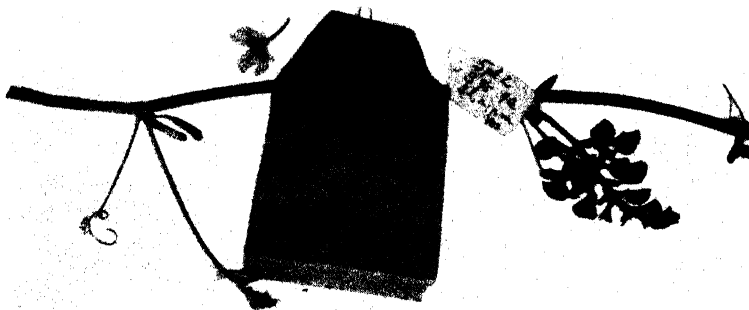


Fig. 5.—Watermelon pollination. Paper bag securely fastened over pollinated pistillate flower with pollinating data on tag, translated as follows: strain 5, plant 2; selfed July 5; 9 a.m.; female, large, receptive; runner medium; yes (meaning that fruit setting is predicted).

In the earlier work, manila paper bags were placed over both types of flowers before anthesis. Although equally effective, their use requires much more time; so, later, cloth bags were used for staminate, paper bags only for pistillate, flowers. In only a very few instances, when the

cloth bags were removed, certain insects were found feeding on the pollen. Still later, cloth bags served for both types of flowers. During seven seasons' work, using cloth bags before anthesis, no evidence of contamination has appeared, even where ten or more varieties, including the inedible citrons, were growing within a few feet of one another. Occasionally ants, beetles, or other insects were found inside the bag at anthesis; but such flowers, even if hermaphroditic, were destroyed.

Although these cloth bags expedite the work under interior California conditions during June, July, and August, they might prove less satisfactory in localities where heavy dews fall at night. Under these circumstances, within the writer's experience in Iowa, certain molds sometimes develop on the stigma after pollination, causing rot or blight. Paper bags, in such cases, are more likely to shed water.

In the earlier work, camel's hair brushes were used for all pollinations; they had to be dipped in alcohol and allowed to dry before being used again. This practice necessitated the employment of a pollinating kit and increased the amount of work necessary for each pollination. At present, all pollinations, whether "selfs" or "crosses," are made by removing the staminate flower with the thumb and finger, care being taken that no part of the hand makes contact with either pollen or stigma. Because of the precautions taken, no contamination has appeared during four seasons, and the amount of labor necessary for each pollination has been materially decreased.

## METHODS AND MATERIALS

Unless otherwise specified, all results here presented were secured with inbred strains of Klondike. This variety is said to have originated as a mutation in a watermelon field in southern California about 25 years ago. Most of the seed used in 1930 had been inbred for four generations, that in 1931 for five, and that in 1932 for six. Presumably, the homozygous nature of the plants used makes the results more reliable than with commercial stocks. This fact is particularly significant with respect to self-sterility, relation between weather and fruit setting, yield per plant, and pollination technique.

The distance between rows was 9 feet and that between hills in the row, 6 feet. Surface-disinfected seed was used, and the resulting plants were thinned to three, later to two, and finally to one per hill. *Diabrotica* beetles were controlled by the use of calcium arsenate mixed with lime (1:20); an occasional infestation of aphids was checked by the use of nicotine dust. With these precautions, approximately perfect stands

were secured during the three years. Normal rate and frequency of irrigations were employed. Unless otherwise specified, the trials were replicated to overcome soil heterogeneity. The plants began to bloom in June (fig. 6), and the first ripe melons were harvested in late July or early August.

At harvest, or very soon after, record was made of individual fruit weight to the nearest half pound; of fruit shape, based on the equatorial and polar diameter measurements to the nearest half inch; of skin color



Fig. 6.—Watermelon pollination, showing progress made within four days after pistillate flowers began to open. When the paper bags are removed, on the second day after pollination, wooden stakes indicate the location of the pollinated flower. Periodic examination of these pollinated flowers provides the data on fruit setting.

and indentation; of rind thickness and toughness; of flesh color, texture, solidity, and relative sweetness (by taste); and of seed size and seed-coat color. From the data on individual fruits, each plant and each inbred strain was arbitrarily rated.

Throughout these investigations a simple pedigree system, initiated by the late Dr. J. T. Rosa, at this station, has been used. Each variety was designated by a number, Klondike being No. 39. Strains designated as 39-5 and 39-9 indicate that selfed melons were secured from plants 5 and 9, respectively, of variety 39. Strains 39-5-3 and 39-5-3-2 were inbred two and three generations, respectively. The original seed of variety 39 was selected by Dr. Rosa from a bulk lot produced by a seedsman at Modesto in 1923. At that time, black-seeded Klondike strains seemed to be in demand; and annual selections for this character have been made (fig. 7).

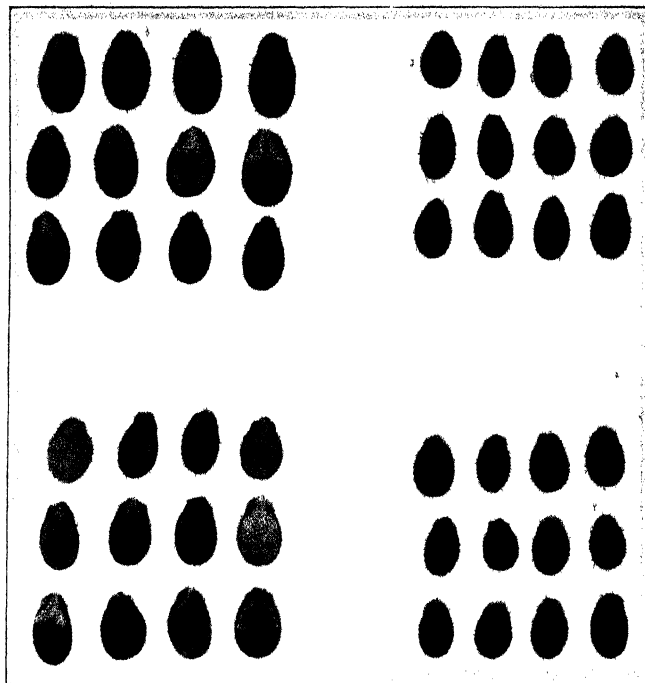


Fig. 7.—Variation in size and color of seeds produced by commercial stocks, left; by California Klondike 1, upper right; and by California Klondike 9, lower right.

## EXPERIMENTAL RESULTS

In 1930, the investigations were confined to measurement of the effects of inbreeding; commercial stocks were compared with strains inbred for one, two, three, and four generations. Particular attention was directed to plant vigor, number of fruits and average yield per plant, and such fruit characters as date of maturity, size, shape, color, rind thickness, flesh color, and texture, as well as seed size and seed-coat color. In Imperial Valley, strains that produce relatively small, early-maturing fruits are much in demand, because the crop from this district begins to ripen in May with a high price per pound; growers have found it easier to sell a melon averaging 18 pounds rather than 25. In the San Gabriel, San Fernando, San Joaquin, and Sacramento valleys, however, the need for a small, early-maturing melon is less, for the crop begins to ripen much later than in Imperial Valley. Growers in these districts prefer a melon averaging 22 to 25 pounds and need more uniform stock of higher flesh quality than is now generally available.

## PLANT VIGOR

In 1930, the strains indicated in table 1 were compared with respect to relative vigor of the plants. As it was physically impossible to measure accurately the leaf area or runner length, measurements of relative plant vigor consisted in observing the rate of plant growth during the season. An arbitrary classification was established as indicated in table 1. Relative vigor was recorded on June 6, July 1, and

TABLE 1

EFFECT OF CONTINUED INBREEDING ON PLANT VIGOR IN WATERMELONS; 1930

Pedigree	Number of generations inbred	Relative vigor*			
		June 6	July 1	August 19	Average for the season
Klondike (commercial).....	.....	1.7	2.4	2.8	2.32
39-5.....	1	2.4	2.3	2.8	2.51
39-5-3.....	2	2.3	2.4	3.0	2.53
39-5-3-2.....	3	2.1	2.3	3.0	2.47
39-5-3-2-9c.....	4	2.4	2.4	3.0	2.62
39-5-12a.....	2	2.3	2.6	3.0	2.71
39-5-12a-2.....	3	2.8	2.3	3.0	2.70
39-5-12a-2-1.....	4	2.6	2.5	2.8	2.69
39-5-3-3.....	3	2.2	3.1	2.5	2.45
39-5-3-3-1.....	4	1.8	2.3	3.0	2.48
39-9.....	1	2.5	2.6	3.0	2.70
39-9-3.....	2	2.0	2.3	3.0	2.43
39-9-3-2.....	3	2.5	2.2	3.0	2.55
39-9-3-2-2.....	4	2.5	2.5	3.0	2.66
39-9-3-4.....	3	2.7	2.4	3.0	2.72
39-9-3-4-9a.....	4	2.8	2.4	3.0	2.79

\* Relative vigor of the unit 1 indicates a comparatively slow-growing vine; the unit 2 shows more thrifty growth than 1; 3 is most thrifty of all.

August 19; the figures were averaged for each strain with the results presented in the table. Obviously, this type of measurement is rather crude; but the data are presented to indicate that there was, to the eye at least, very little difference in relative plant vigor between the various inbred strains and the commercial stock.

Similar observations and records were likewise made during 1931 and 1932, with practically identical conclusions, even though in one strain fruits matured earlier than in others. This early-maturing strain was no more vigorous; but, as will be shown later, the number of days between blooming and fruit maturity was less than with others.

Three years' observation, therefore, indicates that inbreeding of the Klondike does not tend to reduce plant vigor. On the contrary, the vines



of individual inbred strains were more uniform in rate of growth than those of commercial stocks, where both extra-vigorous and relatively slow-growing vines were found. Apparently inbreeding tends to establish homozygosity of those factors responsible for rate of plant growth.

### NUMBER OF FRUITS PER PLANT

Except in a few instances, indicated below, no attempt was made actually to count fruits produced by individual plants, because, when harvesting began, the vines were so intermingled that considerable injury would have resulted. The relatively large number of fruits produced and the number of replications contribute to the accuracy of the results secured. All mature fruits were included, except those infected with blossom-end rot (*Pythium*) or otherwise malformed.

*Results in 1930.*—Commercial Klondike produced 6.3 fruits per plant (table 2), while, with the exception of 39-5-3-2-9, the inbred strains averaged from 6.7 to 9.9. The significance of these differences cannot be statistically determined because the actual number of fruits per plant is unknown; however, with such a large total involved per strain, some of the differences are probably significant. At least, inbreeding evidently did not diminish the number of fruits produced per plant; the tendency was for an increase.

When fruits are sold at a certain price per pound, the total weight per plant is probably just as important as the total number. Late in the season, however, fruits are often sold not by the pound, but at a certain price apiece; and the number of fruits per plant then assumes considerable economic importance. An increased number of fruits per plant does not necessarily indicate an increase in the total weight per plant: decrease in individual fruit weight might tend to diminish total yield even with substantial increase in number of fruits.

Though the inbred strains, in general, tended to produce more fruits per plant than commercial stock, considerable variation appeared among the strains of the former group. Strain 39-5 produced 8.5, strain 39-5-3 produced 8.3, strain 39-5-3-2 produced 7.5, and strain 39-5-3-2-9 produced only 6.2 fruits per plant, indicating a gradual reduction with continued inbreeding. Strain 39-5-3-3 produced 8.0 while 39-5-3-3-1 produced 9.9 fruits per plant; and whereas 39-5-12 produced 6.7, strain 39-5-12-2 produced 7.9, and 39-5-12-2-1 produced 7.6 fruits per plant. With continued inbreeding in the 39-9 strain, the number of fruits per plant varied only slightly. Apparently, therefore, the average number per plant after continued inbreeding, may be extremely variable.

TABLE 2  
YIELD AND TYPE OF FRUITS PRODUCED BY INBRED KLONDIKE STRAINS COMPARED WITH COMMERCIAL KLONDIKE, 1930

Pedigree	Number of generations inbred	Number of fruits produced	Average number of fruits per plant	Yield in pounds		Weight per fruit in pounds		Shape index	
				Per plant	Difference	Mean	Difference	Mean	Difference
Klondike (commercial)	0	189	6.3	121.8	.....	19.6±0.25	.....	645±5.9	.....
39-5	1	236	8.5	138.1	+16.3	16.8±0.17	-2.8±0.302	851±5.9	+206±8.344
39-5-3	2	215	8.3	130.9	+9.1	16.2±0.16	-3.4±0.297	818±6.5	+173±8.772
39-5-3-2	3	150	7.5	142.6	+20.8	19.3±0.27	-0.3±0.369	670±8.0	+25±9.941
39-5-3-2-9	4	155	6.2	125.3	+3.5	19.9±0.27	+0.3±0.369	627±3.8	-18±7.799
39-5-12	2	188	6.7	106.7	-15.1	16.2±0.17	-3.4±0.302	946±6.4	+301±8.653
39-5-12-2	3	119	7.9	145.1	+23.3	19.4±0.27	-0.2±0.369	1,034±3.9	+389±7.003
39-5-12-2-1	4	210	7.6	120.9	-0.9	16.3±0.18	-3.3±0.308	774±8.9	+129±10.71
39-5-3-3	3	76	8.0	110.7	-11.1	12.4±0.18	-7.2±0.308	583±6.1	-62±8.487
39-5-3-3-1	4	249	9.9	131.4	+9.6	13.6±0.11	-6.0±0.273	604±4.2	-41±7.242
39-9	1	225	7.1	125.6	+3.8	18.8±0.22	-0.8±0.333	751±6.4	+106±8.633
39-9-3-2	3	197	7.0	127.2	+5.4	20.3±0.21	+0.7±0.326	569±3.6	-76±8.232
39-9-3-2-2	4	196	7.3	128.6	+6.8	18.8±0.18	-0.8±0.308	548±3.8	-97±6.989
39-9-3-4	3	221	7.4	139.9	+18.1	19.2±0.21	-0.4±0.326	608±3.2	-37±6.712
39-9-3-4-9	4	209	7.8	145.9	+24.1	19.3±0.22	-0.3±0.333	614±3.2	-31±6.712

TABLE 3  
YIELD AND TYPE OF FRUITS PRODUCED BY INBRED KLONDIKE STRAINS COMPARED WITH COMMERCIAL KLONDIKE, 1931

Pedigree	Average number of fruits per plant	Yield in pounds		Weight per fruit in pounds		Fruit shape index	
		Per plant	Difference	Mean	Difference	Mean	Difference
Klondike (commercial)	4 1	72 9	+ 6 2	17 8±0 41	-0 6±0 52	639± 6 3	-46±10 5
39-5-3-2-1-1	4 6	79 1	+11 7	17 2±0 32	+0 6±0 62	583± 8 4	-32±10 2
39-5-3-2-1-2	4 6	84 6	+10 3	18 4±0 47	+0 3±0 41	607± 8 1	-37± 9 5
39-5-3-2-5-1	4 6	83 2	+10 8	18 1±0 07	+0 4±0 56	602± 7 1	-50±12 4
39-5-3-2-5-2	4 6	83 7	- 9 8	18 2±0 38	+1 3±0 74	589±10 7	-49±12 3
39-5-3-2-5-3	3 2	63 1	+16 3	19 1±0 62	+1 6±0 72	590±10 6	-29± 9 05
39-5-3-2-6-1	4 6	89 2	-11 0	19 4±0 59	-0 6±0 72	610± 6 5	-41± 9 1
39-5-3-2-6-2	3 6	61 9	+ 6 2	17 2±0 59	-0 5±0 58	598±10 6	-51±10 2
39-5-3-4-2-1	4 6	79 1	+ 6 7	17 2±0 37	+1 2±0 65	588± 8 0	-14±12 1
39-5-4-3-5-1	4 6	79 6	-12 1	17 3±0 41	+1 0±0 52	625±10 3	-71± 8 9
39-5-12-2-3-1	3 2	60 8	+35 3	19 0±0 50	+2 5±0 71	598± 7 9	-43±10 1
39-5-3-2-5-4	5 6	105 2	+16 4	18 8±0 34	+1 8±0 57	600± 6 8	-28±12 4
39-5-3-2-5-5	4 4	89 3	+44 7	20 3±0 57	+1 8±0 39	617±10 7	-39± 9 3
39-5-3-2-6-3	6 0	117 6	+12 7	19 6±0 39	+0 8±0 59	600± 6 8	-54±10 9
39-5-3-2-9-1	4 6	85 6	0	18 6±0 43	-0 4±0 57	585± 8 8	-60±11 9
39-5-4-1-9-1	4 2	72 9	-20 4	17 4±0 39	+1 4±0 69	579±10 1	-25±14 9
39-5-4-3-0-1	3 2	52 5	-19 1	16 4±0 56	-1 6±0 51	614±13 6	-65±16 1
39-5-12-1-1-1	3 2	51 8	-25 5	16 2±0 33	+2 0±0 63	574±14 8	-75±18 9
39-5-12-2-4-3	3 0	47 4	- 5 6	15 8±0 48	-0 9±0 96	564±17 9	-71±15 6
39-5-12-2-6-1	3 6	67 3	- 2 8	18 7±0 81	-1 1±0 54	598±14 3	-113±11 5
39-5-12-5-0-3	4 2	70 1		16 7±0 36		526± 9 7	

TABLE 4  
YIELD AND TYPE OF FRUITS PRODUCED BY INBRED KLONDIKE STRAINS COMPARED WITH COMMERCIAL KLONDIKE; 1932

Pedigree	Strain No.	Average number of fruits per plant	Yield in pounds		Weight per fruit in pounds		Fruit shape index	
			Per plant	Difference	Mean	Difference	Mean	Difference
Klonlike (commercial)	...	4 1	70 8	.....	17 27±0 261	.....	606±3 09	.....
39-5-3-2-1-1-5	1*	5 0	75 2	+ 4 4	14 86±0 265	-2 41±0 388	601±5 15	- 5±6 33
39-5-3-2-1-2-2	2	4 5	73 3	+ 2 5	16 26±0 299	-1 01±0 409	562±3 50	-44±5 08
39-5-3-2-5-1-1	3	4 3	82 9	+12 1	19 21±0 358	+1 94±0 584	548±5 39	-58±6 54
39-5-3-2-5-2-5	4	4 8	86 1	+15 3	17 88±0 295	+0 61±0 407	557±6 21	-49±7 22
39-5-3-2-5-3-2	5	4 5	82 5	+11 7	18 33±0 365	+1 06±0 588	552±6 41	-54±7 45
39-5-3-2-6-1-5	6	5 1	88 1	+17 3	17 18±0 298	-0 06±0 401	571±5 41	-35±6 56
39-5-3-2-6-2-2	7	4 2	73 7	+ 2 9	17 07±0 364	-0 20±0 587	573±5 80	-33±6 87
39-5-4-3-6-1-18	8	3 6	68 5	- 2 3	19 38±0 402	+2 11±0 400	604±5 90	- 2±6 96
39-5-4-3-6-1-19	9	3 3	58 6	-12 2	17 20±0 361	-0 07±0 586	609±6 40	+ 3±7 43
39-5-4-3-6-1-21	10	2 9	54 5	-16 3	18 92±0 349	+1 65±0 449	599±5 50	- 7±6 61

\* In 1931, the ten best strains were assigned the permanent numbers given in this column.

*Results in 1931.*—Whereas, in 1930, strains inbred for one to four generations were compared with commercial stocks, in 1931 commercial stocks were compared with 20 strains inbred for five generations. As in 1930, inbred strains tended to yield more fruits per plant than commercial stock, this being true of 13 of the 20 strains tested (table 3). The average yield per plant of commercial stock was significantly less in 1931 than in 1930. Extremely hot weather during July, 1931, apparently tended to interfere with fruit setting. The inbred strains varied considerably, but inbreeding evidently did not tend to decrease consistently the number of fruits per plant.

*Results in 1932.*—Though only 10 inbred strains were compared with commercial stocks in 1932, the exact number of melons produced by each of the 237 plants was recorded; and in table 4 in the column headed "Average number of fruits per plant," the figures represent the computed mean. Of the 10 strains tested, 7 produced more fruits per plant than commercial stock, again indicating that many inbred strains produce as many as commercial stock, or more.

*Discussion.*—According to the data above, our present seed supply of inbred Klondike strains may be safely increased and distributed through seed trade channels without danger of decreasing the number of fruits per plant. In fact, statistics<sup>(23)</sup> show that for watermelons the average number of fruits for the United States in 1930 was only 322 per acre. During the same period (table 5), the average number in Imperial Valley was 654, while in other districts in California it was 775 per acre.

TABLE 5  
NUMBER OF WATERMELON FRUITS PER ACRE FOR THE IMPORTANT DISTRICTS  
OF THE UNITED STATES

State	Number of fruits per acre*			
	1928	1929	1930	Average
Georgia.....	300	340	350	330
Florida.....	275	288	273	279
Texas.....	250	180	235	222
South Carolina.....	300	330	325	318
California.....	691	732	720	714
Alabama.....	250	320	380	317
Missouri.....	286	272	190	249
North Carolina.....	300	180	270	250

\* Data taken from a published summary<sup>(23)</sup> of the Bureau of Agricultural Economics, United States Department of Agriculture.

With a planting distance of 9 by 6 feet and thinning to one plant per hill, 660 plants may be produced on one acre. Since many growers thin

to 2 plants per hill, with 1,320 plants per acre they should be able to produce 2,500 fruits. With improved Klondike strains the average for California might well be increased to 1,000 fruits per acre. In an extensive planting in Imperial Valley in 1932, comparing commercial stock with 10 of our inbred strains, a significant difference noted by the cooperator was that the inbred strains produced relatively few cull fruits. Experience at Davis has shown this observation to be correct. Inbreeding and selection tend to eliminate inferior plants and to allow for gradual improvement within the variety. Seedsmen could well afford to devote considerable attention to watermelon breeding.

### TOTAL YIELD IN POUNDS PER PLANT

The total yield per plant, in pounds, is determined both by the weight and by the number of the fruits. Some small-fruited varieties such as Winter Queen and Baby Delight (Hungarian Honey) are commonly known to produce many more fruits per plant than such large-fruited varieties as Tom Watson and Thurmond Grey. The fact that a plant produces relatively few melons does not necessarily indicate a low total yield.

*Results in 1930.*—As it was physically impracticable to record the actual yield of each plant separately in 1930, the data represent only mathematical averages. Of 14 strains under comparison (table 2), 11 produced a greater total yield than the commercial stock, and only 2 of the remaining 3 produced significantly less. There is, furthermore, no consistent evidence that continued inbreeding of a particular strain causes any significant decrease in total yield. In the progeny of 39-9, a gradual increase in average plant yield appeared with continued inbreeding, even though average fruit weight remained fairly constant. In the inbred progeny of strain 39-5, an additional generation of inbreeding sometimes lowered and sometimes increased total yield per plant. Evidently, then, total plant yield, expressed in terms of fruit weight, was not significantly lowered by inbreeding.

*Results in 1931.*—Average yield per plant in 1931 was determined in the same manner as in 1930 (table 3). Of 20 strains tested, 11 produced a higher and 8 a lower yield than commercial stock. The 5 progeny of strain 39-5-12 consistently produced a lower yield; and two strains, 39-5-12-1-1-1 and 39-5-12-2-4-3, also produced fruits significantly smaller than commercial stock. These strains have been discarded. With two exceptions, the progeny of strain 39-5-3-2 consistently produced a high average plant yield; and several seed com-

panies are now increasing the seed supply of this strain for distribution as California Klondike.

*Results in 1932.*—The progeny of strains 39-5-3-2 and 39-5-4-3 were compared with commercial stock in 1932 (table 4), but the yield of each plant was exactly determined by individual records. As the data show, the progeny of the former strain yielded more and that of the latter strain less than commercial stock. Since the progeny of strain 39-5-4-3 tend to mature fruit somewhat earlier than any others, inbreeding of this strain will be continued, its fruit being also of desirable type, size, and quality for Imperial Valley conditions.

### AVERAGE WEIGHT PER FRUIT

As previously stated, growers in Imperial Valley prefer a smaller fruit than those in districts where the watermelon crop matures later. Often a relatively small-fruited strain produces more melons per plant than one larger fruited; hence there might be no significant decrease in total yield per plant even though the melons were of smaller size. An ideal Klondike type for Imperial Valley conditions would be a strain producing fruits of uniform type and high quality and maturing three to four per plant with an average weight of 18 to 20 pounds. In districts where the fruit ripens later than in Imperial Valley, the same general type is desired; but growers prefer a strain producing an average fruit weight of 22 to 25 pounds.

*Results in 1930.*—As each melon was weighed separately, the average weight could be ascertained and statistical formulas applied in order to evaluate the difference between inbred strains and commercial stock.

From the original data for each strain were prepared class-frequency tables showing the weight of individual fruits. The means were then computed. The standard deviation as well as the probable error of the mean was determined by the usual method, and the probable error of the differences was computed. The difference in mean weight was considered significant only when at least three times the probable error of the difference.

The data derived from the 1930 trials, as presented in table 2, indicate a general tendency for the mean fruit weight of inbred strains to fall below that for commercial stock. This decrease was significant in only 6 of the 15 strains tested, and 4 of these showed an increase in plant yield because more melons were produced than in commercial stock. Because strains 39-5-12 and 39-5-3-3 produced such small fruits, they and their progeny have been discarded.

Though none of the inbred strains produced heavier fruit than commercial stock, a study of the frequency distribution based on actual fruit weight shows greater uniformity among inbred strains than in

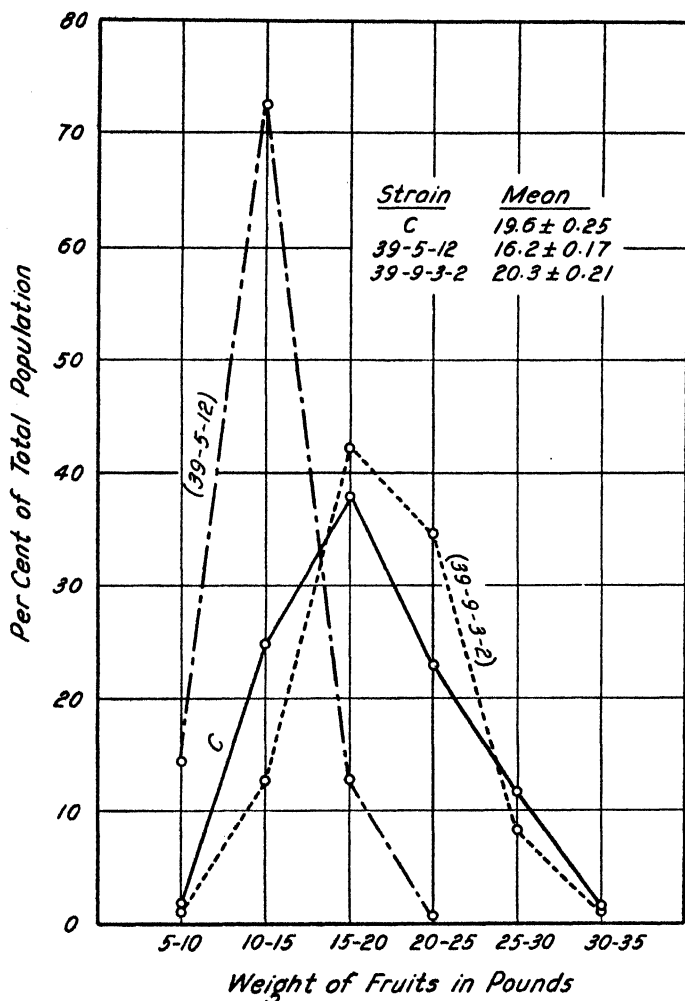


Fig. 8.—Frequency distribution of commercial Klondike, C, and two inbred strains, on the basis of individual fruit weight; 1930 data.

commercial stock. This was marked in 39-5-12, a small-fruited strain, and evident in 39-9-3-2, the largest-fruited strain tested in 1930 (fig. 8). Inbreeding apparently serves to isolate both small and large-fruited strains and tends to establish uniformity with respect to fruit size.



*Results in 1931.*—In 1931, two strains produced significantly heavier and two significantly lighter fruit than commercial stock (table 3). Whereas strain 39-5-12-1-1 has been discarded on account of inferior quality, the seed supply of strain 39-5-4-3-6-1 has been increased for distribution because the fruit seems to mature a few days earlier than among other strains. Though the mean weight of fruits of inbred strains seldom differed significantly from that of commercial stock, statistical examination consistently indicated that the former were much more uniform, with a much smaller standard deviation, than the latter.

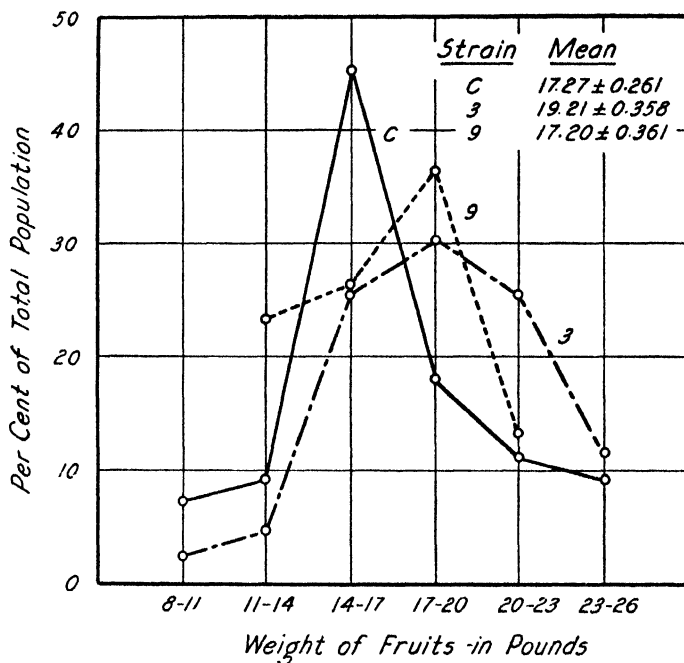


Fig. 9.—Frequency distribution of commercial Klondike, C, and two inbred strains, on the basis of individual fruit weight; 1932 data.

*Results in 1932.*—The strains tested in 1932 had been inbred for six generations (table 4). Of 10 inbred strains, 3 produced heavier and only 1 lighter fruit than commercial stock. Almost without exception, the fruits of the former were much more uniform in weight, than the latter. The frequency distribution of strains 3 and 9 is compared in figure 9, with commercial stock C, indicating the more pronounced fruit weight uniformity among the inbred strains. At present, strain 3 seems to be the most desirable of the many inbred strains for seed increase and distribution to the trade. It will be later described in detail.

*Discussion.*—On the basis of mean fruit weight in combination with many other desirable fruit and vine characteristics, the seed supply of strains 1 and 2, each inbred for six generations, will be increased and distributed to the trade with a recommendation for truck gardening and home use. Although somewhat small for commercial use in the important districts of the state, the fruits are of extremely high quality. Strain 3 appears ideal for Imperial Valley conditions, being of desirable weight, shape, and quality, with satisfactory yield per plant. When tested in Imperial Valley in 1932 in competition with nine other inbred strains and commercial stock, it manifested qualities desirable for that district. At Davis in 1932, one plant of strain 3 produced seven mature melons, weighing 16, 16, 23, 23, 24, 25, and 26 pounds respectively, an average weight of 21.7 pounds. As three of these melons resulted from artificial self-pollination this new strain will probably continue to produce large fruit and be adapted to districts where fruit matures later than in Imperial Valley.

### FRUIT-SHAPE INDEX

The lack of desirable fruit-shape uniformity in commercial Klondike stock has led many growers to conclude that this variety is “running out” with continued culture in California. In some districts this lack of uniformity has led to the substitution of certain other varieties lower in flesh quality than Klondike. In commercial fields, quite diverse fruit types are common, some long and slender, others decidedly oval to nearly round. Whether this variation results entirely from segregation or from varietal mixtures is not known, but certainly we need strains that will produce fruits of more uniform shape.

Fruit-shape index was determined by dividing the equatorial by the polar diameter. Individual fruits were measured to the nearest half inch. Thus, if a fruit measured 16 by 8 inches, the shape index (ignoring the decimal point) was 500. Desirable fruit-shape index for the Klondike variety lies between 500 and 625. If less than 500, the fruit is considered too slender; if more than 625, too blocky. During these investigations, fruits have been found with shape indexes as low as 388 (18 by 7 inches) and as high as 1,333 (9 by 12 inches). Inbreeding tends to isolate these undesirable types and permits their elimination. Fruits of uniform weight and shape are easily loaded for shipment and reach the market with minimum bruising and breakage. They also make a more attractive display.

*Results in 1930.*—The fruit-shape index of fourteen inbred strains is presented in table 2. With three exceptions, the differences were sig-

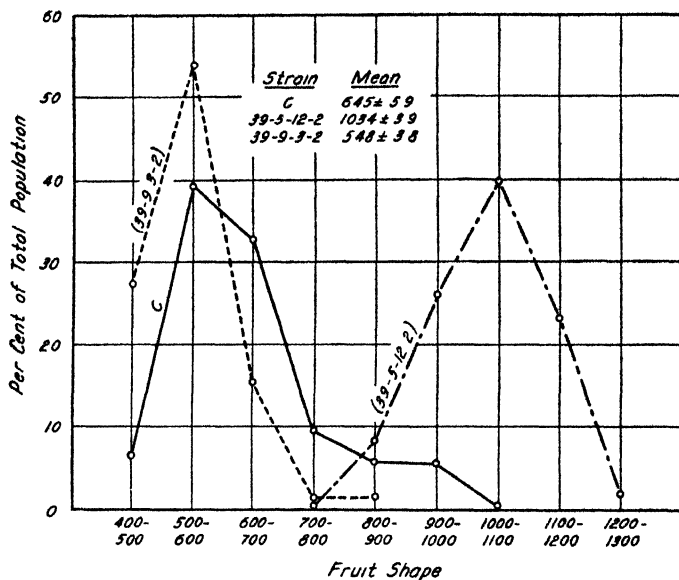


Fig. 10. - Frequency distribution, based on the ratio of equatorial to polar fruit diameter, of commercial Klondike, C, and two inbred strains; 1930 data.

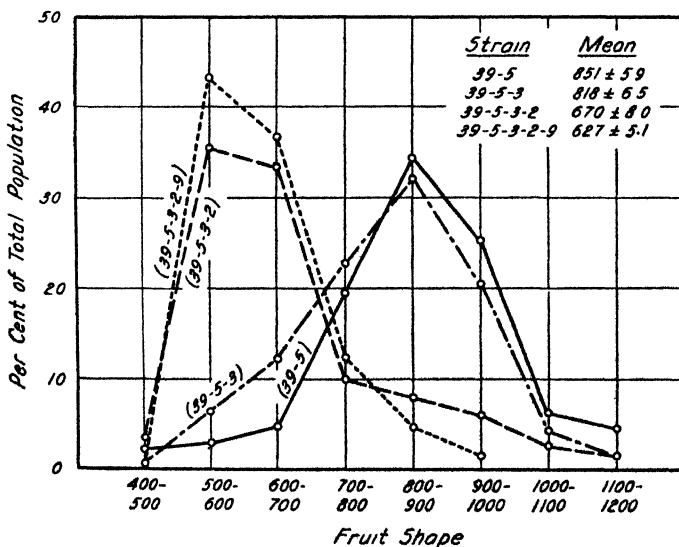


Fig. 11.—Frequency distribution, according to fruit shape, of strains inbred for one, two, three, and four generations, showing the effects of selection toward oblong fruits; 1930 data.

nificant. One should note that within strains 39-5 and 39-9, selections were purposely made for oblong fruit shape. With continued inbreeding of strain 39-5 to 39-5-3-2-9 and with conscious selection of oblong fruits, the fruit-shape index decreased from  $851 \pm 5.9$  to  $627 \pm 3.8$ . Four generations of inbreeding, with particular attention to desirable fruit shape, tended to eliminate the undesirable types. With respect to fruit shape, among commercial stocks and two strains (39-5-12-2 and 39-9-3-2) each inbred for three generations, figure 10 indicates that this character was becoming fixed. The spread among commercial stock varied from 400 to 1,100, that of 39-5-12-2 from 700 to 1,300, and that of 39-9-3-2 from 400 to 900.

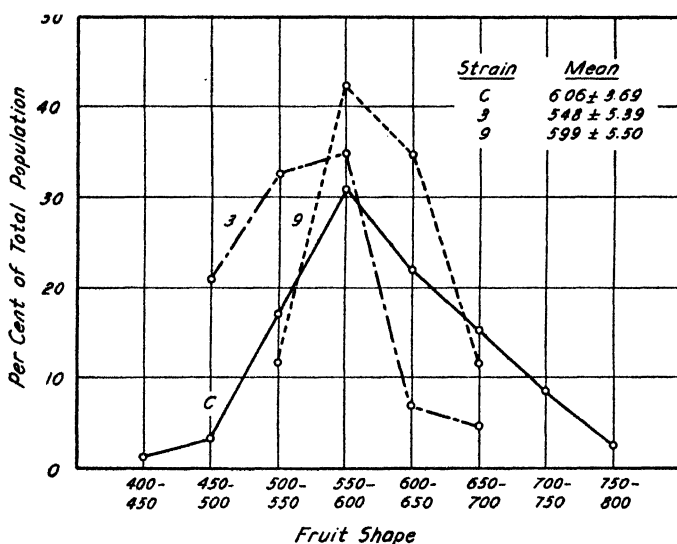


Fig. 12.—Frequency distribution, according to fruit shape, of commercial Klondike, C, and strains 3 and 9; 1932 data.

The frequency distribution of 39-5, 39-5-3, 39-5-3-2, and 39-5-3-2-9, as indicated in figure 11, shows that continued selection and inbreeding for four generations tended to stabilize fruit shape. The spread of 39-5 was from 400 to 1,200 (mean  $851 \pm 5.9$ ); that of 39-5-3-2-9, from 400 to 1,000 (mean  $627 \pm 5.1$ ), with 80 per cent of the fruits indexing between 500 and 700.

*Results in 1931.*—Of 20 strains tested, all were of more desirable fruit shape than commercial stocks (table 3). The differences were less pronounced than in 1930, because all the inbred strains had been inbred for five generations, with selection for desirable type. All the strains, in fact, showed a fruit-shape index between 500 and 625. Undesirable fruit types had been eliminated.

*Results in 1932.*—The ten strains tested in 1932 had been inbred for six generations (table 4), and all were within the range of desirable fruit shape. Six strains differed significantly from commercial stocks in fruit shape, all being more desirable. In figure 12, commercial stock *C* shows a range from 400 to 800 (standard deviation 70.9); strain 3(39-5-3-2-5-1-1) from 450 to 700 (standard deviation 54.2); and strain 9(39-5-4-3-6-1-22) from 500 to 700 (standard deviation 42.1). According to these data, six generations of inbreeding, with elimination of undesirable fruit types, served to fix the character for desirable type. As indicated earlier, strain 3 has proved well adapted to commercial production.

## DISCUSSION OF INBREEDING EFFECTS AND RESULTS

Inbreeding of Klondike watermelons, therefore, does not consistently lessen plant vigor, number of fruits, nor total yield per plant, and may serve to isolate strains that produce fruits of weight and type equal or superior to commercial stocks. The practice of inbreeding does not serve to create new strains; rather, it improves the stocks by uncovering certain undesirable types that should be eliminated.

One should note that certain inbred strains seem actually to have lost vigor, as expressed in terms of weight and number of fruits per plant. Such strains have been discarded, for the purpose of our breeding work is primarily to improve commercial stocks, not to examine the genetic constitution of the inbred strains. Certain strains, however, now inbred for six generations, are no less vigorous than commercial stock. The seed supply of such strains has been or will be increased for distribution to growers and seedsmen, with the assurance that it will produce more uniform fruits of higher flesh quality than commercial stock and will yield equally well. Evidently, furthermore, in commercial stocks, relatively few of the genetic factors governing plant vigor exist in the heterozygous condition, for the inbred strains tend to fluctuate only slightly from the yield of commercial stocks. If a large number of the vigor factors were in the heterozygous condition, yield fluctuations would probably be greater.

No data have been presented to indicate the effects of inbreeding on fruit-skin color; on rind thickness and toughness; on flesh color, texture, solidity, and sugar content; nor on seed size or seed coat color. Obviously considerable diversity exists among these characters, and though certain of them have received well-merited consideration, their uniformity and variation among inbred strains could not always be measured accurately.

The late Dr. Rosa at first made particular effort to improve flesh quality and at the same time to incorporate the black-seeded character. Though all the more desirable inbred strains now produce black seeds, this character is not considered so important as formerly. The seeds of the inbred strains are significantly smaller, with more per pound, than in commercial stocks (table 6). This character is of some importance, for it increases the acreage that may be planted with a certain quantity

TABLE 6

VARIATION IN NUMBER OF SEEDS PER POUND AMONG COMMERCIAL  
AND INBRED KLONDIKE WATERMELON STRAINS

Year grown	Place grown	Stock	Seeds per pound
1930	Los Angeles	Commercial	6,019
1930	Los Angeles	39-5-3-2-9-op*	11,222
1931	Los Angeles	Commercial	8,799
1931	Modesto	Commercial	7,149
1932	Los Angeles	Commercial	5,748
1932	Los Angeles	39-5-4-3-6-1-3	11,604
1932	Los Banos	Commercial	6,864
1932	Los Banos	39-5-3-2-1-1-5	9,243
1932	Modesto	39-5-3-2-5-op-op	8,962
1932	Modesto	39-5-4-3-6-1-6	8,853
1932	Davis	Commercial	7,113
1932	Davis	39-5-3-2-1-1-op	8,449
1932	Davis	39-5-4-3-6-1-18	9,265

\* The designation "op" means open-pollinated.

of seed. With respect to flesh characters, much uncontrollable variation exists because not all fruits can be harvested at the same stage of maturity. Hence the uniformity of this character has been measured, relatively, by a large number of fruits of all strains. The importance of a rind strong enough to withstand shipping has been realized; and, though the most desirable inbred strains produce relatively thin rinds ( $\frac{1}{4}$  to  $\frac{1}{2}$  inch), tests have shown that the fruits will endure rough handling with minimum breakage.

## DESCRIPTION OF IMPROVED STRAINS

Cooperative field tests of the more desirable inbred strains, with commercial stocks as checks, have been made annually since 1930. These tests have been conducted with growers and seedsmen in Imperial, Riverside, Los Angeles, Kern, Tulare, Stanislaus, San Joaquin, and Butte counties. The advisability of these widely separated tests is obvious.

Though the description of the strains is based, primarily, upon their comparative response at Davis, these outlying tests have often brought to light some desirable and some undesirable characters not evident here. The most complete of the outlying tests have been conducted in the Imperial Valley for two reasons: first, hundreds of acres there are not yet severely infested with the wilt organism (*Fusarium niveum*); and second, during 1928, 1929, and 1930 approximately 62 per cent of the state's watermelon acreage was located there.

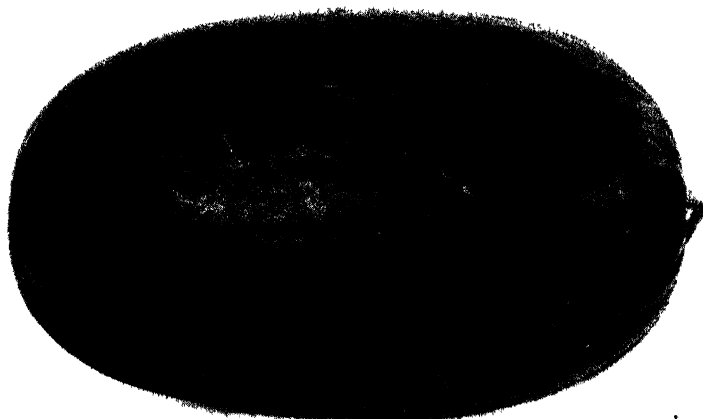


Fig. 13.—A typical mature fruit of California Klondike strain No. 3. Weight, 20 pounds; shape index 581 (15.5 × 9 inches); skin dark green; suture slight; rind thickness  $\frac{3}{8}$  inch; flesh deep red, of excellent texture and high sugar content; seeds small, with black seed coat.

In order to avoid confusion, the improved strains are designated as California Klondike, and seed has been and will be distributed under this varietal name. A brief description of four strains of the California Klondike watermelon follows (table 4).

*Strain 1.*—Relatively small fruited; average weight 15 pounds; shape index, 601; skin dark green and smooth, with very slight suture; rind very thin ( $\frac{1}{4}$  to  $\frac{3}{8}$  inch) and somewhat brittle; flesh deep red, of excellent texture and high sugar content; seeds small and black; very prolific; recommended to market gardeners and for home planting; fruits too small and rind too tender for extensive commercial use.

*Strain 3.*—Relatively large fruited; average weight, 19 pounds; shape index 548 (fig. 13); skin very dark green with shallow suture; rind of medium thickness ( $\frac{1}{4}$  to  $\frac{1}{2}$  inch), sufficiently tough for long-distance shipment; flesh very solid, deep red, of excellent texture and high sugar

content; seeds small and black; prolific; uniform, producing very few culls; one plant of this strain produced seven ideal fruits in 1932, with an average weight of 21.6 pounds; recommended to commercial planters wherever the Klondike is now grown, and to market gardeners who prefer a larger melon than strain 1.

*Strain 8.*—This strain, though somewhat inferior in flesh quality, might well replace Angeleno as a shipping melon to such distant points as Canada. Ideal type with deep bluish-green skin and extremely tough rind. Flesh slightly hard, but sweet.

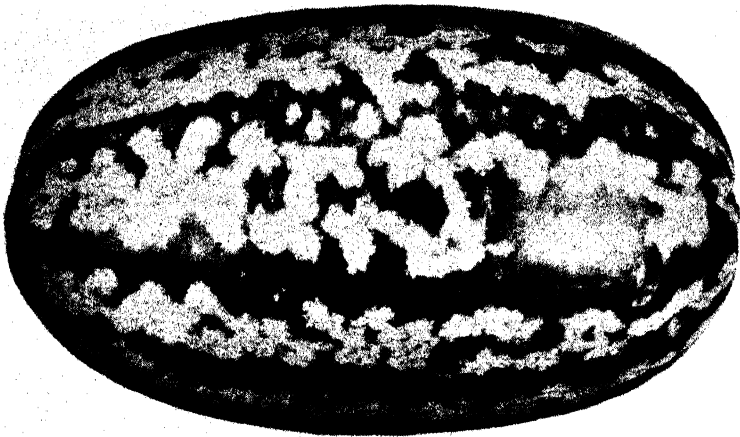


Fig. 14.—Striped Klondike, a relatively recent commercial selection, at present gaining in popularity in the San Joaquin and Sacramento valleys.

*Strain 9.*—Fruits of this strain, in 1931, appeared to mature earlier than any other strain both in the Imperial Valley and at Davis. Seedsmen report it to be early maturing, but this point should be more definitely ascertained. The fruit is of desirable type and quality, and seed is available.

*Striped Klondike.*—This variety (fig. 14), now advertised by certain seed companies, is said to have been originally selected by a grower in interior California about ten years ago in a field of commercial Klondike. Continued mass selection by growers for the striped-skin character, large fruit, and high flesh quality has isolated a strain remarkably uniform as to skin color. At Davis, in 1932, approximately 97 per cent of the plants of this variety produced striped fruit. Fruit size and shape, rind thickness, flesh quality, and seed characters varied somewhat; but this is to be expected in a variety not kept pure by continued self-



pollination. At Davis, the flesh quality was somewhat inferior to that of the same variety grown on lighter soil types in San Joaquin and Stanislaus counties, and was slightly inferior to ordinary commercial Klondike. As Striped Klondike seems to be increasingly popular, breeding work to purify it further is now under way.

## CONDITIONS INFLUENCING FRUIT SETTING

Knowledge of the conditions that influence fruit-setting tendencies in *Citrullus vulgaris* is limited chiefly to field observations. Growers operating in humid districts feel that fruit setting is hindered by rainfall occurring between 6 and 11 a.m. Those in arid districts appreciate the injurious effects of high air temperature (above 100° F), low air humidity (below 20 per cent), high wind velocity, and extreme sunlight intensity. In Iowa under humid conditions, the effects of some of these factors, in relation to fruit-setting tendency after artificial self-pollination, often appeared worthy of investigation. Opportunity for the studies reported herewith presented itself in the course of the breeding work in California, where no rain fell during pollination and where considerable variation in forenoon air temperature existed.

## AIR TEMPERATURE AND FRUIT SETTING

Comprehensive investigations of the relation between air temperature and fruit setting were initiated in 1931, after the experience in 1930 had strongly indicated that fruit setting might have been influenced by abnormally high temperatures during the morning hours.

*Results in 1931.*—Artificial self-pollination of 10 Klondike strains, each inbred for five generations, began on June 20 and continued until July 11. Pollination of the same strains, planted considerably later, began on August 3 and terminated on August 16. The date, time, and result of each pollination were recorded. Obviously, not all the selfed flowers set fruit—the number borne by individual plants is much too high; but with a relatively large number of flowers involved, and with random selections made each day, the results have probably some significance.

The results of this study appear in table 7, showing the number of pollinations made each day, and the percentage that set fruit, with corresponding air temperatures during the morning hours, from 8 to 10, 10 to 12, and 8 to 12 inclusive. Of 435 self-pollinations made during June and July, only 70 (or 16.1 per cent) set fruit. The percentage fruit

setting, per day, varied from 0.0 in seven instances to as high as 33.3 on June 21. For purposes of comparison, attention is directed to results secured between June 24, when 32, and July 8, when 53 flowers were selfed. Comparison of percentage set on each day with that on the day immediately preceding or following, does not always indicate that hot

TABLE 7  
WEATHER CONDITIONS IN RELATION TO FRUIT SETTING IN INBRED  
KLONDIKE WATERMELONS; 1931

Date		Pollinations		Average air temperature, degrees Fahr.			Relative per cent humidity of the air at 12 m.
		Number	Per cent successful	8 a.m. to 10 a.m.	10 a.m. to 12 m.	8 a.m. to 12 m.	
June	20	9	0 0	60	70	64	37
	21	9	33.3	62	75	67	47
	22	16	0 0	69	82	74	34
	23	17	0 0	66	79	71	26
	24	32	18.7	82	85	84	23
	25	25	0 0	83	98	89	20
	26	43	13.9	84	94	88	23
	27	22	22.7	75	82	78	35
	28	32	31.2	68	80	73	35
	29	25	8 0	76	85	80	14
30	24	4 1	78	88	82	17	
July	1	20	10 0	83	92	87	17
	2	17	11.8	80	94	86	26
	3	23	26 1	87	97	91	15
	4	18	0 0	92	102	96	12
	5	2	0 0	86	104	93	22
	6	7	0 0	81	89	85	26
	8	53	24.5	84	94	88	22
	9	14	21.4	82	95	87	17
	10	16	31.3	86	96	90	14
	11	11	27.3	80	95	88	21
Average		....	16.1	79	91	83	24
August	3	7	28.5	70	83	75	36
	4	2	100.0	68	84	74	40
	5	7	42.8	70	84	76	35
	6	9	22.2	68	80	72	36
	7	12	41.6	64	82	71	36
	8	12	41.6	66	82	72	31
	9	11	27.2	69	84	75	33
	10	13	38.4	65	80	71	36
	11	8	25.0	73	86	78	34
	12	11	54.5	78	89	82	24
	13	15	20.0	79	87	82	32
	14	11	18.1	68	80	73	44
	15	9	33.3	71	84	77	30
	16	2	50.0	80	96	87	23
	Average		....	36.6	71	84	76

TABLE 8  
EFFECT OF AIR TEMPERATURE AND HUMIDITY ON FRUIT SETTING, IN INBRED KLONDIKE WATERMELONS; 1932

Date	Number of pollinations	Per cent successful pollinations	Hourly and average air temperatures, degrees Fahr.								Hourly and average air humidity, per cent									
			6 a.m.	7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12 m.	Mean 6 a.m. to 12 m.	Mean 12 m. to 6 p.m.	6 a.m.	7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12 m.	Mean 6 a.m. to 12 m.	
			6 a.m.	7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12 m.	Mean 6 a.m. to 12 m.	Mean 12 m. to 6 p.m.	6 a.m.	7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12 m.	Mean 6 a.m. to 12 m.	
5 .....	4	0.0	76	84	87	94	95	97	97	90	99	46	39	36	31	26	26	33	37	
6 .....	1	100.0	51	55	59	64	70	76	87	63	100	75	68	65	60	50	37	32	55	25
7 .....	4	25.0	54	57	61	66	76	84	92	70	103	72	67	64	54	46	42	34	53	25
8 .....	24	50.0	56	64	70	76	87	94	97	78	105	62	55	47	26	12	12	12	32	21
9 .....	22	54.5	58	64	70	74	76	78	77	71	77	64	58	54	54	52	50	48	54	51
10 .....	23	65.2	45	50	56	60	64	66	70	59	81	67	64	60	55	50	37	27	51	29
11 .....	34	58.8	50	50	53	57	64	67	71	59	80	73	67	62	54	45	40	36	54	35
12 .....	55	52.7	48	53	58	64	70	75	82	64	81	67	63	60	53	36	34	34	49	41
13 .....	117	61.6	52	52	54	56	60	63	66	58	76	75	72	67	65	60	55	50	63	46
14 .....	154	58.4	51	56	60	66	73	78	82	67	88	70	65	60	48	43	38	35	51	34
15 .....	88	40.9	55	62	68	75	80	84	87	73	90	63	60	50	44	39	33	25	45	32
16 .....	93	38.7	53	58	65	73	76	83	87	71	93	68	63	55	47	40	32	25	47	30
17 .....	84	50.0	54	57	64	70	76	84	87	70	85	70	65	58	45	35	35	35	49	40
18 .....	49	47.0	54	55	56	62	65	67	71	61	71	73	70	66	65	60	55	55	63	53
19 .....	2	100.0	46	53	57	64	70	76	81	64	91	74	68	62	54	37	27	27	50	25
20 .....	0	.....	54	57	67	80	84	87	92	74	100	63	42	35	31	26	23	20	34	25
21 .....	3	33.3	52	57	64	73	80	86	94	75	95	66	50	52	35	29	19	19	39	26
22 .....	7	85.5	52	57	63	72	76	83	87	70	94	70	66	55	47	40	32	31	49	32
23 .....	3	0.0	52	56	65	72	77	85	89	71	95	67	62	55	46	40	33	24	47	28
Averages.....	....	51.8	53	58	63	69	75	80	84	69	90	68	61	56	48	40	35	31	48	35

weather reduced fruit setting. The entire period was abnormally warm, with many desiccating winds of high velocity. Attention, however, is called to comparative fruit setting in relation to temperature on June 24 and 25, June 26 and 27, June 28 and 29, and July 3 and 4.

Between August 3 and 16, inclusive, 129 pollinations were made, of which 35.6 per cent set fruit. The air temperature during this period was lower than during the pollinating period in June and July, and the respective averages were also lower.

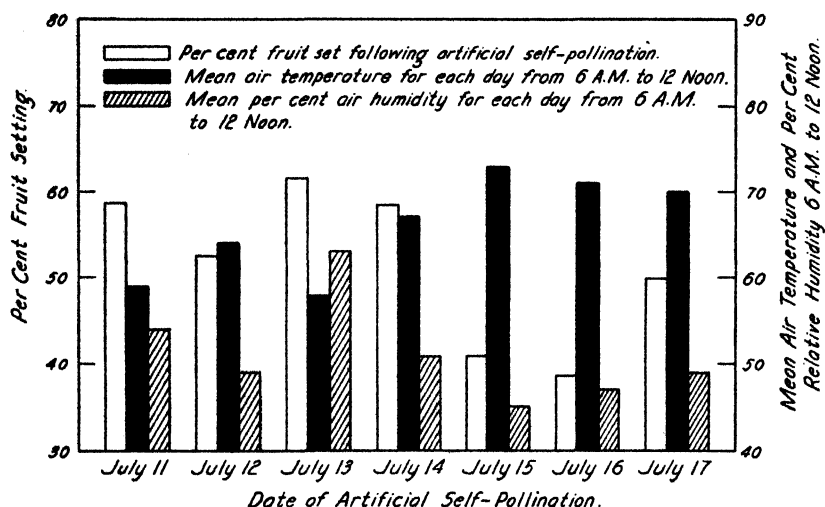


Fig. 15.—The apparent relation of air temperature and air humidity from 6 a.m. to 12 noon, to rate of fruit setting after artificial self-pollination of inbred Klondike strains at Davis, 1932.

*Results in 1932.*—Investigation of the temperature effects in 1932 were continued more extensively than in 1931, although all pollinations were made during 19 days, from July 5 to 23, inclusive. The means for the periods 6 a.m. to 12 noon, and 12 noon to 6 p.m. are listed in table 8. Marked variation in temperature occurred throughout the pollinating period: that at 6 a.m. varied from 45° to 76° F, that at 9 a.m. from 56° to 94°, and that at 12 noon from 66° to 97°. In several instances, high percentage set was directly related to low average air temperature from 6 a.m. to 12 noon. This relation is clearly indicated in figure 15, where the data for each day are compared with those for both the preceding and following day. The highest percentage set (61.6) occurred on July 13, when the temperature at 12 noon was only 66° F and the average for the morning was only 58° F, the lowest during the 19-day period. On 10 different days the average air temperature from 6 to 12 was higher,

and on 8 days it was lower than the average (69° F) of the entire pollinating period. During the former group, the set was 44 per cent; during the latter, 58 per cent, again indicating that fruit-setting tendency was greater during cool weather.

## AIR HUMIDITY AND FRUIT SETTING

Under arid climatic conditions, the relative humidity of the air varies considerably from hour to hour during the day and from day to day. At Davis, this is particularly true during the summer months, when the humidity may be reduced from 62 per cent at 6 a.m. to 12 per cent by 12 noon. Such decided fluctuations might affect fruit-setting tendencies.

*Results in 1931.*—Although hourly record of air humidity was not made in 1931, table 7 shows the actual humidity at 12 noon for each day of the pollinating period, with the forenoon temperature for the early and late pollinating periods. During the June and July pollinating period the mean humidity at noon was 24 per cent, while the percentage set was 16.1. During the August period the mean humidity increased to 34, the set to 35.6.

*Results in 1932.*—Air humidity was recorded hourly during the pollinating period; and the various means, indicated in table 8, were determined. The percentage humidity at 6 a.m. on the different dates varied from 46 to 75; at 9 a.m. from 26 to 65; and at noon from 12 to 55. The mean humidity from 6 a.m. to 12 noon varied from 33 to 63; that from 12 noon to 6 p.m., from 21 to 53.

The relation of high percentage set to high relative humidity is more evident when only the morning hours are considered. Apparently the air humidity after 1 p.m. exerts little influence upon fruit setting.

TABLE 9

AIR TEMPERATURE AND HUMIDITY IN RELATION TO FRUIT SETTING IN KLONDIKE WATERMELONS; 1931 AND 1932

Period	Total self-pollinations	Per cent fruit set	Air temperature, degrees Fahr.			Relative humidity, per cent		
			Forenoon	Afternoon	24-hour period	Forenoon	Afternoon	24-hour period
June 20 to July 11, 1931.....	435	16.1	83	93	84	26	31	29
August 3 to 16, 1931.....	129	35.6	76	87	80	34	32	33
July 5 to 23, 1932	767	51.8	69	90	78	48	33	40

Between July 11 and 17, inclusive, high percentage set was almost constantly related to high relative humidity, this in turn (fig. 15) being correlated with low air temperature. Apparently, low air temperature and high relative humidity jointly encourage high percentage set after selfing. The interrelations of temperature, humidity, and fruit-setting tendency are shown in table 9, where these conditioning factors for 1931 and 1932 are compared. The data show that the morning temperature was lower and the humidity higher during 1932 than in 1931, with a corresponding increase in fruit-setting percentage in 1932.

### FRUIT SETTING AS INFLUENCED BY TIME OF POLLINATION

With each self-pollination, record was made of the hour, vigor of runner, size of ovary, and receptivity of stigma. During 1931, pollinations were made between 6 a.m. and 1 p.m. Table 10 summarizes the data for 1931 and 1932. During June and July, 1931, fruit setting showed a very slight, probably insignificant tendency, to increase

TABLE 10  
TIME OF DAY AT WHICH SELF-POLLINATIONS WERE MADE IN RELATION TO FRUIT  
SETTING IN KLONDIKE WATERMELONS

Time of day	Trial* No.	Total pollinations	Total fruits set	Percentage of fruits set
6 to 7 a.m.....	1	13	2	13.1
7 to 8 a.m.....	1	152	22	14.5
8 to 9 a.m.....	1	220	37	16.8
9 to 10 a.m.....	1	78	13	16.7
10 to 11 a.m.....	1	17	3	17.6
6 to 7 a.m.....	2	7	2	28.6
7 to 8 a.m.....	2	23	8	34.8
8 to 9 a.m.....	2	34	15	44.1
9 to 10 a.m.....	2	35	16	35.5
10 to 11 a.m.....	2	34	15	44.1
6 to 7 a.m.....	3	77	36	46.7
7 to 8 a.m.....	3	168	85	50.6
8 to 9 a.m.....	3	177	95	53.7
9 to 10 a.m.....	3	176	73	41.5
10 to 11 a.m.....	3	113	45	39.8
11 to 12 a.m.....	3	70	29	40.1
12 to 1 p.m.....	3	8	0	0.0
6 to 9 a.m.....	3	422	216	51.2
9 a.m. to 12 m.....	3	359	147	40.9

\* Trial 1 extended from June 20 to July 11, 1931; trial 2 from August 3 to 16, 1931; and trial 3 from July 6 to 22, 1932.

between 6 and 11 a.m. In 1932, it increased slightly between 6 and 9, with a gradual decrease in efficiency by 1 p.m. The average percentage set, in 1932, between 6 and 9 a.m. was 51.2; that between 9 and 12, only 40.9 per cent. Fertilization appears most likely to follow self-pollinations made between 7 and 11 a.m.

### OVARY SIZE IN RELATION TO FRUIT SETTING

In the absence of experimental evidence, observation has indicated that fruit-setting tendency is influenced by ovary size. In 1932, therefore, as each fruit was pollinated, its relative size was recorded, being arbitrarily classified as small, medium, large, or very large. Periodically during the season each selfed fruit was examined, and record was made

TABLE 11  
RELATION OF OVARY SIZE AT TIME OF POLLINATION TO FRUIT SETTING IN  
KLONDIKE WATERMELONS; 1932

Relative size of ovary at pollination	Total self- pollinations	Total fruits set	Percentage of fruits set
Small.....	70	5	7.1
Medium.....	363	154	42.4
Large.....	300	163	54.3
Very large.....	58	41	70.7

of all unsuccessfully fertilized. At harvest time, those that matured fruit were likewise noted. Data covering these observations appear in table 11; they indicate that small ovaries rarely set fruit, while fruit-setting tendency progressively increases among larger ovaries. Considerable time, apparently, could be saved by careful selection of the flowers to be pollinated.

### VIGOR OF RUNNER IN RELATION TO FRUIT-SETTING TENDENCY

The relative vigor of the individual runner bearing the pistillate flowers to be selfed was recorded. According to the data in table 12, fertilization is much more likely to occur if the fruits selected are borne on medium and strong runners rather than weak. As a matter of fact, weak runners have rarely been observed to produce large or very large pistillate flowers. Here again, time would be saved by selecting only medium or strong runners.

TABLE 12

STRENGTH OF RUNNERS BEARING FEMALE FLOWERS IN RELATION TO FRUIT SETTING  
IN KLONDIKE WATERMELONS; 1932

Relative strength of runner at time of pollination	Total self- pollinations	Total fruits set	Percentage of fruits set
Weak.....	67	19	28.4
Medium.....	381	145	38.0
Strong.....	327	168	51.4

## FRUIT-SETTING TENDENCY AND EARLINESS AMONG INBRED STRAINS

Although certain strains consistently produce more fruits per plant than others, as has been demonstrated, there is no evidence that fruit-setting tendency, after self-pollination, is heritable. During 1931 and 1932, the percentage set was recorded for each of ten inbred strains, with the results presented in table 13. As previously indicated, the percentage

TABLE 13

FRUIT SETTING AND ELAPSED TIME TO FRUIT MATURITY AFTER POLLINATION IN  
KLONDIKE WATERMELONS, COMPARING INBRED STRAINS, 1931, WITH  
THEIR RESPECTIVE DAUGHTER STRAINS, 1932

Pedigree	Percentage of fruits set		Number of days between pollination and fruit maturity	
	1931	1932	1931	1932
39-5-3-2-1-1.....	22.7	.....	42	....
39-5-3-2-1-1-5.....	.....	50.6	....	45
39-5-3-2-1-2.....	29.8	.....	43	....
39-5-3-2-1-2-2.....	.....	56.5	.....	47
39-5-3-2-5-1.....	19.1	.....	45	....
39-5-3-2-5-1-1.....	.....	47.9	.....	48
39-5-3-2-5-2.....	13.3	.....	47	....
39-5-3-2-5-2-5.....	.....	58.3	.....	50
39-5-3-2-5-3.....	13.5	.....	49	....
39-5-3-2-5-3-2.....	.....	60.6	.....	50
39-5-3-2-6-1.....	19.1	.....	49	....
39-5-3-2-6-1-5.....	.....	60.9	.....	51
39-5-3-2-6-2.....	5.0	.....	44	....
39-5-3-2-6-2-2.....	.....	54.7	.....	48
39-5-3-4-2-1.....	19.3	.....	44	....
39-5-3-4-2-1-3.....	.....	46.8	.....	48
39-5-4-3-6-1.....	15.2	.....	39	....
39-5-4-3-6-1-18.....	.....	27.9	.....	43
39-5-4-3-6-1-19.....	.....	54.5	.....	41
39-5-4-3-6-1-21.....	.....	17.6	.....	45
39-5-12-2-3-1.....	16.3	.....	49	....
39-5-12-2-3-1-6.....	.....	41.2	....	53



set in 1931 was extremely low, probably because of unfavorable climatic conditions. In that year the set in strain 39-5-3-2-6-2 was only 5, while that in strain 39-5-3-2-1-2 was 29.8 per cent. The progeny of both strains, when replanted in 1932, set fruit at almost exactly the same rate. Similar instances indicate that fruit-setting tendency is probably nonheritable, although some strains normally bear more fruits per plant than others.

Since the number of days between anthesis and fruit maturity was known, data were assembled during both seasons, for each strain, to indicate the relative earliness of each. The data in table 13 show that the time between anthesis and fruit maturity varied, in 1931 from 39 to 49 days, and in 1932 from 41 to 53 days. Strain 39-5-4-3-6-1-19 does not bloom earlier but has matured fruit somewhat earlier than other inbred strains. In 1931, it was approximately 10 days earlier than commercial stock in Imperial Valley; but in 1932, under more extreme conditions, this earliness was much less apparent.

#### VARIETAL AND HYBRID INFLUENCES ON FRUIT-SETTING TENDENCY

Slight differences in fruit-setting tendency were found during both seasons, when several varieties and hybrids were compared (table 14). In 1931, the percentage set varied from 12.1 (new crosses) to 25.0 (Yellow-fleshed Ice Cream). In 1932, however, 56.9 per cent of the new crosses set fruit. The most notable difference in 1932 was between California Klondike and Striped Klondike. In the former variety 51.8 and in the latter only 33.2 per cent of the selfed flowers set fruit. The Striped Klondike had not been inbred, whereas the California Klondike had been inbred for six generations.

Self-pollinations have been made among the following varieties: California Klondike, Striped Klondike, Dixie Belle, Peerless, Golden Honey, Yellow-fleshed Ice Cream, Chilean, Angeleno, Baby Delight (Hungarian Honey), Georgia Rattlesnake, Thurmond Grey, Tom Watson, Pride of Muscatine, Iowa King, Iowa Belle, Grey Monarch, Wondermelon, Schochler, Winter Queen, and Black Boulder. A large number of crosses of the three wilt-resistant varieties Pride of Muscatine, Iowa Belle, and Iowa King with California Klondike, as well as numerous crosses involving many other varieties, have been successful. Fruit setting appears to depend upon proper selection of the pistillate flower with respect to ovary size and runner vigor, as well as upon the environmental conditions for a period of 5 or more hours after the pollination has been made.

Critical examination of fruit-setting tendency on individual plants of the Klondike variety has indicated the complete absence of either definite flowering peaks or definite fruiting cycles, such as those reported in *Cucumis melo* by Rosa,<sup>(18)</sup> in *Lactuca sativa* by Jones,<sup>(8)</sup> and in *Cucurbita maxima* by Bushnell.<sup>(2)</sup>

TABLE 14  
FRUIT-SETTING TENDENCY OF SEVERAL WATERMELON VARIETIES  
AND HYBRIDS, 1931 AND 1932

Varietal designation	Year	Number of generations inbred	Total pollinations	Per cent set
California Klondike.....	1931	5	564	20.6
California Klondike.....	1932	6	767	51.8
Striped Klondike.....	1932	0	226	33.2
Dixie Belle.....	1931	3	43	18.8
Dixie Belle.....	1932	4	65	47.7
Peerless.....	1931	1	40	20.0
Peerless.....	1932	2	27	66.6
Golden Honey.....	1931	3	21	23.8
Golden Honey.....	1932	4	35	51.5
Yellow-fleshed Ice Cream.....	1931	2	16	25.0
Yellow-fleshed Ice Cream.....	1932	3	24	58.3
F <sub>1</sub> of 8 crosses.....	1932	.....	95	46.3
New crosses*.....	1931	.....	33	12.1
New crosses.....	1932	.....	51	56.9
Back crosses.....	1931	.....	22	13.9
Back crosses.....	1932	.....	48	41.9

\* Involving crosses of wilt-resistant × wilt-susceptible varieties as well as inter-crosses within the Klondike variety.

## SUMMARY

Although the watermelon (*Citrullus vulgaris*) is typically monoecious, andromonoecism is common to several varieties. Parthenocarpy probably does not occur, and neither self nor cross-sterility has been encountered among the many varieties and hybrids studied.

The ratio of pistillate to staminate flowers is approximately 1:7, but the former are not always formed at every seventh node on a runner.

Pollinating technique is described, with a discussion of its adaptation under various environmental and cultural conditions.

Plant vigor, expressed in terms of vegetative growth, is not reduced by four generations of inbreeding; and no striking vigor reduction has been observed in strains inbred for six generations. Inbreeding tends to equalize and stabilize individual plant vigor.

Inbreeding tends to isolate strains producing more or fewer fruits per plant than commercial stock, but the variation in average fruit weight often compensates for reduction in number of fruits per plant.

Strains have been isolated that produce fruits of either greater or less average weight than commercial stock, and with significant decrease in standard deviation.

Total plant yield, determined by average fruit weight and number of fruits per plant, may be slightly increased or decreased by inbreeding, indicating that relatively few of the factors which govern yield are in a heterozygous condition in commercial stocks.

The commercial Klondike was found to be extremely heterozygous for the factors governing fruit shape, expressed in terms of the ratio between equatorial and polar diameter. Inbreeding permits the elimination of strains of undesirable shape, leaving genotypes significantly more uniform than commercial stock. Similarly, the standard deviation with respect to fruit shape is lowered.

Inbreeding tends to purify individual strains with respect to: fruit skin color; rind thickness and toughness; flesh color, texture, solidity, and sweetness; seed size and seed-coat color.

Several inbred strains are described with recommendations as to their adaptability to certain sections and to certain uses. California Klondike No. 1 is recommended to the market gardener or home grower; No. 3 to growers in Imperial Valley and in any other section where the Klondike is now used; No. 8 for long-distance shipment; and No. 9 for trial in Imperial Valley because of some evidence that its fruit matures earlier than commercial stock.

Fruit-setting tendency, following artificial self-pollination, is apparently influenced by air temperature and humidity between 6 a.m. and 12 noon; by the hour of pollination; by ovary size; and by relative vigor of the runners bearing the selfed flowers.

Indications are that relatively low air temperature and relatively high air humidity from early morning until noon favor fruit setting. On certain days when the morning temperature was relatively high and the humidity relatively low, none of the selfed flowers set fruit.

In general, a slightly higher percentage of pollinations results in fruit setting if made between 6 and 9 a.m. than if made between 9 a.m. and 12 noon.

The fruit-setting tendency was relatively low when small ovaries were selected, but relatively high if large ones were used. Very few fruits set if the runner bearing the pistillate flower was weak; but with stronger runners the fruit-setting tendency materially increased.

With the exception of Striped Klondike, all the varieties used responded to self-pollination in approximately the same manner. Definite flowering peaks have not been detected.

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# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 7

SEPTEMBER, 1933

No. 16

## METHODS OF BREEDING ONIONS<sup>1</sup>

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Breeding studies with the onion were begun at Davis in 1923. At first, the object was to develop a more uniform strain of Australian Brown. As the work progressed, however, the program was gradually expanded. At present, besides the improvement work with this variety, attention is being given to the development of onions that will resist pink root, mildew, and thrips; to the selection of nonbolting strains of Sweet Spanish; and to studies of the way in which the scale and flesh color, foliage color, shape, and other characters are inherited. In all this breeding work, special methods have had to be developed. Those that have proved best at Davis may need modification in other districts. At Davis, climatic conditions are nearly ideal for onion breeding. During May and June, when the plants are in bloom, atmospheric conditions are usually very favorable for pollination: rain seldom falls, the days are generally bright, and there is no dew. The plants, consequently, can be enclosed with very little danger of mildew.

### DEVELOPMENT OF THE INFLORESCENCE AND THE FLOWER

Usually the mother bulbs are planted in late November or in December. Toward the end of February, if the plants are dissected, the flower stalks can be distinguished just above the stem plate. Sometime during March, as a rule, they emerge from the surrounding sheaths. They elongate rapidly, the developing buds being protected under the bracts. Finally the bracts are ruptured, and a few days later the first flower of the inflorescence opens.

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When the two outer whorls (perianth) of the flower first expand, (fig. 1) the pistil is still immature; but soon the stamens begin to shed their pollen. There are six stamens in each flower, an inner and an outer whorl each containing a set of three. Those of the inner whorl are first to

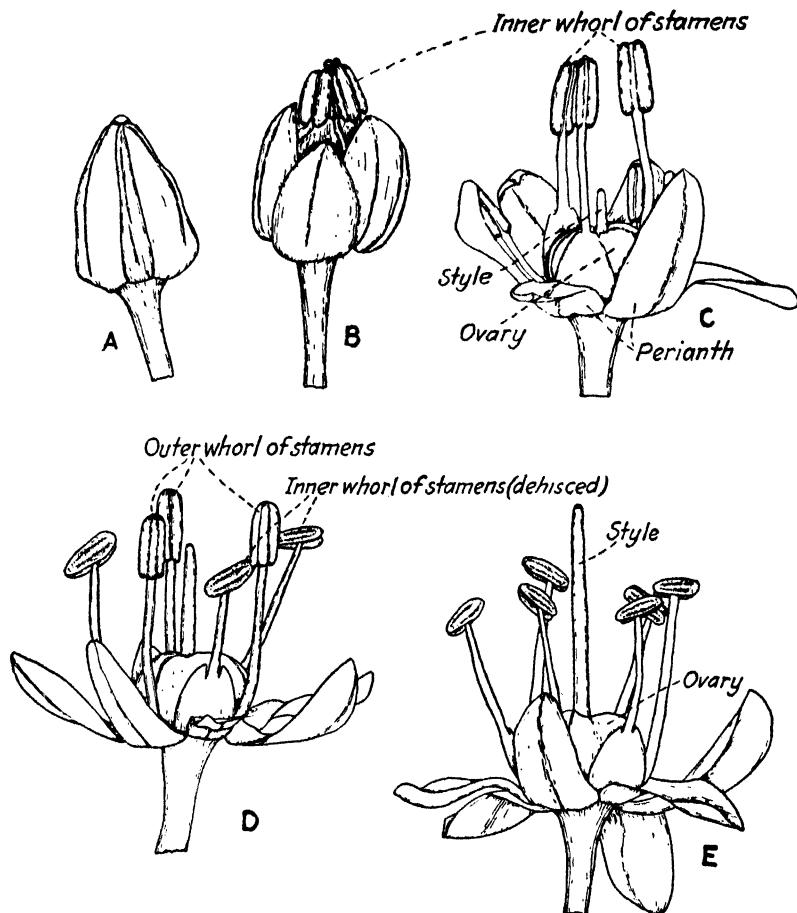


Fig. 1.—Method of flower-opening and pollen-shedding in the onion. *A*, flower bud just before opening. *B*, the two outer whorls of floral organs expanding and the inner whorl of stamens elongating. *C*, just before the shedding of pollen by the inner whorl of stamens; note the short style. *D*, the inner whorl of three stamens has shed its pollen, and the outer whorl of three stamens has elongated. *E*, all six stamens have shed their pollen; note the long style, now receptive. (From *Truck Crop Plants*. McGraw-Hill Book Co., Inc., New York.)

shed their pollen, dehiscing one after the other at irregular intervals. Next, the anthers of stamens in the outer whorl also dehisce at irregular intervals. This is the regular order of dehiscence, though occasionally anthers of the outer whorl discharge their pollen first. All the pollen is

shed before the stigma becomes receptive. When the flower first opens, the style is about 1 mm long. It continues to elongate, but does not reach its maximum length of about 5 mm until some time after all pollen has been shed. The flowers of a single head may continue to open over a period of two weeks or longer.

A detailed study of anther dehiscence was made from June 2 to June 4, in 1923. Fifty flowers on as many different plants were tagged at 4 p.m. on June 2. Each flower had the perianth fully expanded, and the stamens had not dehisced. It was 23 hours before all the anthers of any flower had dehisced, and about 47 hours before shedding of the pollen was completed. Most of the pollen was shed between 9:30 a.m. and 5 p.m.

In the field, cross-pollination probably predominates; but there is some selfing. The chief agencies of pollination are various species of insects, which go from flower to flower, either gathering pollen or visiting the nectaries in the axils of the three inner stamens.

#### METHOD OF HANDLING PLANTS FOR SELFING

The plants to be selfed are usually set in the field sometime in the fall or early winter. Varieties such as California Early Red and Italian Red, which do not keep well in storage, are generally planted in late September or October. The storage types, such as Australian Brown, are usually set in the field in late November or December. Bulbs are spaced about 12 inches in furrows 3 feet apart (fig. 2) and are then covered with soil to prevent freezing. The plants develop a good root system during the winter, but the scantiness or luxuriance of the foliage depends upon climatic conditions. As a rule, the flower stalks emerge from the surrounding sheaths sometime in March, and the first flowers open in early May. The entire inflorescence is then immediately enclosed within a manila paper bag. Varieties with large flower heads, such as California Early Red and Italian Red, are covered with one-pound paper bags, tied closely so that the flowers within are crowded. This practice seems to give a better set of seed, although the point has not been definitely proved. If the bags are small and are tied close to the inflorescence, very little surface is exposed—a very important consideration where strong winds are prevalent during the pollinating season. Square-bottomed bags are used (fig. 3); and the necessary data, such as pedigree and date of bagging, are written on the bottom. The plots are gone over twice each day, and the heads that have open flowers are bagged. By bagging twice each day, one can get the exact date when



each plant blooms; the main purpose, however, is to prevent insects from visiting open flowers and bringing in foreign pollen. Even though the stigmas are not receptive until several days after the flower first

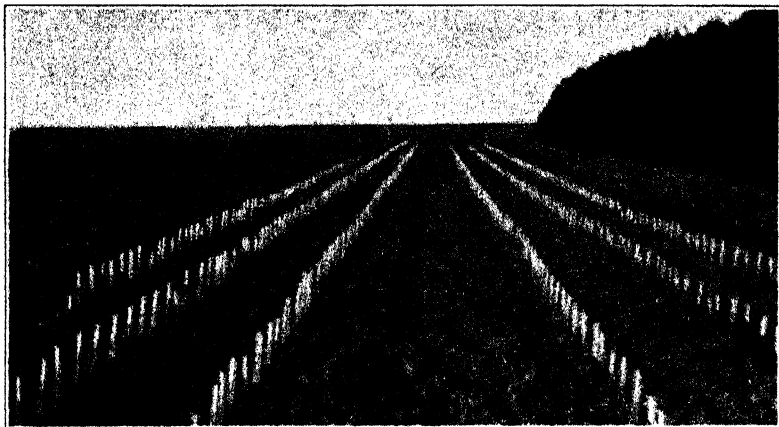


Fig. 2.—All the bulbs that are to be selfed are staked and labeled at the time of planting. The center row is left vacant for a planting of corn. (Photographed February 20, 1928.)



Fig. 3.—Selfing onions at the University Farm, Davis, California. Corn is planted as a windbreak. The tall Italian types of onions are growing in the row on the left. The other two rows are storage types. The field is the same as in figure 2.

opens, visiting insects might possibly leave foreign pollen that could function later. Although the heads may be bagged before the bracts are split, to do so usually results in a lower yield of seed.

During 1923 and 1924 a few tests were made in the field to compare the amounts of seed obtained with different methods of pollination. In 1923 the Australian Brown variety was used. The data, as given in table 1, are not very extensive, but they do show a great reduction in

TABLE 1

POLLINATION STUDIES WITH THE VARIETY, AUSTRALIAN BROWN; 1923

Method of pollination	Number of plants	Number of seed heads	Seed harvested per head
Open-pollinated	9	24	712
Bagged, wind-shaken	9	10	157
Bagged, tied to stake	4	8	54

seed yield when bagged heads are compared with open-pollinated. The Australian Browns used in this experiment were commercial bulbs. The flower heads were covered with one-pound manila paper bags; one lot was allowed to sway freely in the wind but was not tapped by hand; the other was bagged and tied to stakes to prevent movement by the wind. Under bagged conditions, the shaking by the wind apparently tended to increase the seed yield, probably by better distribution of the pollen.

TABLE 2

POLLINATION STUDIES WITH THE VARIETY, EBENEZER; 1924

Method of pollination	Number of plants	Number of heads	Average number of flowers per head	Seed harvested per head	Per cent of perfect set
Open-pollinated	19	45	525	1,000	32
Emasculated, open-pollinated	7	7	498	580	19
Bagged, shaken by hand	10	21	517	205	7
Bagged, windshaken	16	28	525	99	3
Bagged, before bracts broke, wind-shaken	11	23	846	85	2

In 1924, different methods of pollination were further compared, this time with the Ebenezer variety (table 2). Because the number of flowers per head varies widely, the best method of expressing seed yield is as percentage of perfect set—which is six seeds for each flower. By far the heaviest yield of seed was obtained under open-pollinated conditions; but even then there was only a 32 per cent set. Seldom is a 50 per cent set obtained even under ideal circumstances. Emasculated heads gave a much lower set of seed than did those not emasculated, but a much higher set than did any of the bagged heads. The emasculated flowers were fertilized by pollen from other plants or from other flower heads

of the same plant. The lower set of seed from emasculated flower heads indicates that some pollination occurs between flowers of the same head. The lowest set of seed was obtained where the heads were bagged before the bracts covering the flower buds had broken. Shaking or tapping by hand gave the heaviest set of seed under bags.

TABLE 3

SEED YIELD FROM BAGGED PLANTS OF DIFFERENT VARIETIES, AT DAVIS

Year	Variety	Number of plants	Average number of seed stems	Seed yield	
				Per head	Per plant
1923	Yellow Danvers Flat.....	31	6.74	172	1,162
	Red Wethersfield.....	31	4.97	100	497
	Southport White Globe.....	33	3.39	146	496
	White Portugal.....	28	2.86	173	495
	Southport Yellow Globe.....	19	2.58	96	249
	Southport Red Globe.....	20	2.40	42	101
1924	California Early Red.....	28	4.07	258	1,049
	Ohio Yellow Globe.....	14	5.28	171	905
	Red Wethersfield.....	35	6.43	130	834
	Mountain Danvers.....	11	3.27	201	657
	Yellow Globe Danvers.....	80	4.74	121	573
	Australian Brown.....	82	4.40	117	515
	Red Bermuda.....	15	3.27	96	313
	Riverside Sweet Spanish.....	12	5.08	57	292
	Giant White Italian Tripoli.....	32	3.50	76	268
1925	Ebenezer.....	36	4.25	57	244
	Italian Red.....	39	4.44	207	917
	Yellow Danvers Flat.....	39	4.82	63	306
	Southport Yellow Globe.....	30	4.07	70	286
	Ebenezer (Lot 1).....	29	5.27	45	238
	Ohio Yellow Globe.....	37	4.65	47	218
	Yellow Globe Danvers.....	30	4.17	47	198
	White Portugal.....	89	4.25	39	164
	Mountain Danvers.....	14	3.64	33	122
	Southport Red Globe.....	38	3.10	37	116
1926	Southport White Globe.....	58	3.64	32	116
	Ebenezer (Lot 2).....	15	4.40	6	27
	Valencia.....	17	5.53	31	172
1928	Southport Red Globe.....	11	2.73	33	91
	Red Wethersfield.....	8	5.37	42	224
1929	Stockton Yellow Globe.....	37	5.89	174	1,025
1930	Australian Brown.....	197	7.14	219	1,568
1932	Nebuka ( <i>Allium fistulosum</i> ).....	26	3.11*	713	2,222
	Stockton Yellow.....	17	6.94	159	1,106
	Red Wethersfield.....	19	5.00	82	412
	White Persian.....	73	7.55	26	195

\* Only a few of the early flower heads of Nebuka were bagged.

The seed yield per plant, which varies considerably with the variety, is given in table 3 for a number of varieties. The bulbs used were from commercial stocks, selected for selling because of certain outstanding characters. All were large. They were not planted for the purpose of comparing varieties, but obviously some yielded considerably more seed than others. Nonbolting types like Italian Red, California Early Red, Stockton Yellow, and Stockton Yellow Globe usually give a good seed yield under bags. Different strains of the same variety vary considerably. In 1925, one lot of Ebenezer averaged 238 seeds per plant; another strain, only 27. These strains also differed considerably in seed yield under conditions of open-pollination. As a rule, the white varieties yield less seed than the colored. The Nebuka (*Allium fistulosum*) types, which do not form bulbs and which are used by the Japanese as green onions, produce more seed per plant and per head than any of the commonly cultivated bulbing varieties grown in America. Also, because of more favorable climatic conditions, seed yields are much higher in some years than in others. As shown by table 3, 1924 was much more favorable for the production of seeds under bags than was 1925, when intense heat killed many of the young seeds. At Davis, on June 24, 25, and 26, maximum temperatures were 113°, 115°, and 112° F; on July 16 and 17, they were 112° and 116°. The number of seeds produced per plant decreases with inbreeding for several generations, but that matter will be discussed in another paper.

## WINDBREAKS

Temporary windbreaks of corn are grown each year to protect the bagged onions from the prevailing south winds and the occasional strong north winds. In the spring, as soon as the soil is sufficiently warm, corn is planted on the outside of the field (fig. 3) and also in rows left vacant for this purpose (fig. 2).

## METHOD OF INCREASING SEED

When this work was first begun, certain strains or lots of onions that were to be increased were planted in presumably isolated farming districts. Because, however, complete isolation was almost unattainable, some crossing generally occurred. The constant danger of loss, the extensive amount of traveling required, and inability to secure complete isolation necessitated the development of methods that assured control of pollination.

After several preliminary tests, the method of increasing seed under cloth cages was finally adopted, so that all the different lots can now be handled on the University Farm.

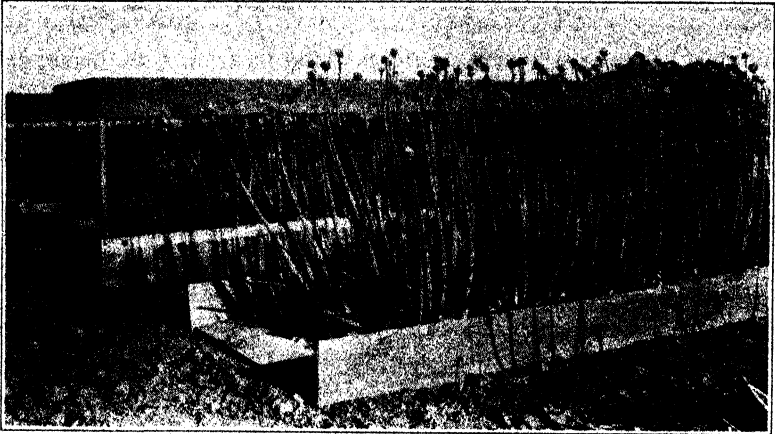


Fig. 4.—Method of planting onions in small groups. The groups are covered just before the first flowers open. This photograph shows a framework for cages being built around several different lines of California Early Red. As the seed stems of this variety are exceptionally tall, a high cage is needed.

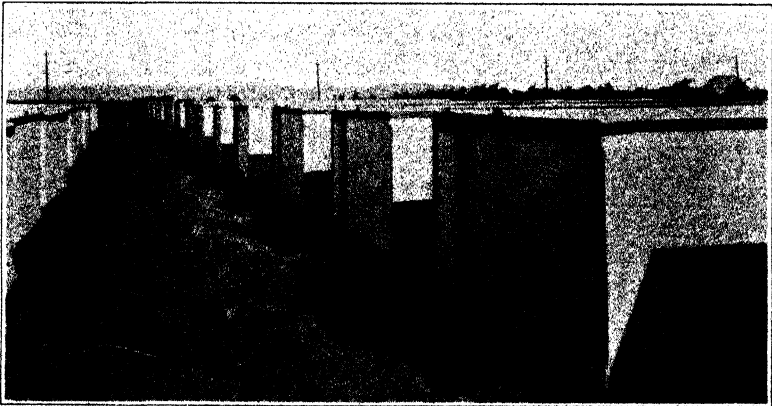


Fig. 5.—Large cheesecloth cages used for increasing seed. The cheesecloth is used for one season only. The same lumber is used year after year.

The bulbs of selections that are to be increased are planted in the field in late November. They are set 4 to 6 inches apart in two shallow parallel furrows about 2 feet apart and 15 feet long. In the spring, just before the plants begin to bloom, they are covered with cages made of a framework of boards upon which cheesecloth is tacked. Stakes are first driven into the ground at the corners and midway along the sides.

A 12-inch board (fig. 4) is then placed around the bottom and sunk in the ground about 2 inches. Around the top and lengthwise through the center, boards are nailed; around the sides is tacked a 3-foot width of cheesecloth (fig. 5). Two widths of the latter material are placed across the top, well lapped, and tacked to the sides and top so as not to pull out. A door is made in one end to permit entrance to the cage. For most varieties a cage 4 feet high is sufficient (fig. 6); but Italian Red,



Fig. 6.—Interior view of a large onion cage about three weeks after enclosing the plants. Variety, White Persian.

California Early Red, and the like, require one 6 feet high. Under Davis conditions, apparently, the best results follow when the large cages are placed at right angles to the prevailing winds; this arrangement probably permits a greater movement of air inside.

Means must be provided for pollination within the cages. Both bees and flies have been tried; but the latter, being more easily handled, have proved more satisfactory. The flies are propagated under controlled conditions in order to insure that adults will be free from foreign pollen. Slaughterhouse refuse, mainly beef lungs, is placed in open troughs. This attracts various species of adult flies, the most prevalent being

*Phormia regina* Meig., the sheep wool maggot, and *Lucilia sericata* Meig., the green blow-fly. They deposit their eggs in large numbers. During onion-pollination time, as a rule, the larvae hatch in 24 to 36 hours and begin to feed. Lungs are an especially good medium, being porous and providing a large feeding area. The trough should be shaded by burlap or muslin in order to protect the larvae from the hot sun. The young larvae, being gross feeders, should be well supplied with food. After feeding for 5 to 7 days, they become restless and wander about in search of a place to pupate. This restless period lasts 4 to 8 days, during which they do not feed. They may be collected at this time by shaking the pieces of lungs so the larvae will fall into the trough. Then they work their way to the end of the trough and drop into a bucket of fine-screened sand, into which they burrow before pupating. After all the larvae have pupated, the pupae are recovered from the sand by screening; then they are washed by running a fine stream of water over the screen and are dried by spreading a thin layer on a newspaper and stirring it frequently. When completely dry, the pupae are placed on a paper plate, on top of which another plate is inverted, the edges being fastened with clips. If cold storage is available, the pupae can be kept for a considerable time. At 45° F the flies emerge in about two weeks; at 37° F they can be kept for several months. If stored at various temperatures, practically mature pupae will be available at all times. A succession of broods should be maintained so that pupae can be added to the cages every three or four days. The time required to produce a generation of flies under natural conditions varies greatly with the season, being considerably longer in the spring than later when temperatures are high.

By starting to propagate the flies early in the year and then holding the pupae in cold storage, one can have a large supply in readiness for the pollinating season. When the plants begin to bloom, a small handful of pupae are placed in the cage every three or four days; thus the number of flies within the cage can be held fairly constant.

A high percentage of the developing seed within the cage is often killed during periods of excessive heat. This may not occur in locations where lower temperatures prevail during this part of the year. At best this method can be used only to increase small amounts of seed. Sufficient seed should be obtained from a cage (fig. 5) to plant  $\frac{1}{8}$  to  $\frac{1}{2}$  acre, and this will furnish sufficient bulbs to plant a considerable acreage for seed.

## CROSSING

The method selected for controlled cross-pollination depends upon several factors. When the hybrids that result from the crossing of two plants can be easily identified, the following method is generally used. Bulbs are set side by side, about a foot apart. Usually two of each kind are planted so that if one is killed or dies, another remains; most of the plants, however, survive. If exact genetic studies are being made, only two parent plants are kept; but if improved commercial types are desired, without regard for genetic records, several plants of each strain

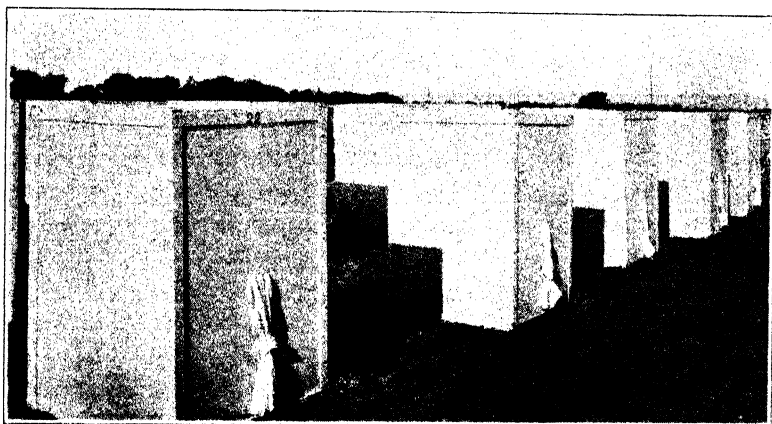


Fig. 7.—Muslin and cheesecloth cages used for crossing. These cages are so constructed that they can be taken apart at the close of the season and stored.

are permitted to grow. These small lots are covered with cages about 3 feet square and 6 feet high (fig. 7). Into one side of the cage is sewed a sleeve through which fly pupae can be introduced. The cages are left in place until the seed is mature. Seed from different strains or parents is harvested separately and later planted separately. In some cases the hybrids can be selected in the seedling stage; in others, the bulbs must be grown.

A few examples will illustrate the amount of crossing that occurs under the cages and the method of selecting hybrids. In 1931 there were placed under one of the small cages two bulbs of Stockton Yellow (inbred one generation) and a single bulb of Italian Red (inbred four generations). The Italian Red is bottle-shaped; the Stockton Yellow, flat. In the seedling stage, because red is dominant, it is possible to select the hybrids among the progeny of the yellow parent, but not among the



progeny of the Italian Red. Because, however, the mature bulbs are intermediate in shape between the two parents and very much larger, the hybrids in the population from each parent can be selected accurately. Part of the seed from the Stockton Yellow parent was planted, and a total population of 315 plants was grown. Of this number, 76 (about 24 per cent) were crosses with the Italian Red. Some seed of the Italian Red parent was planted, and a population of 282 plants was grown. Of this number, 79 (about 28 per cent) were crosses with Stockton Yellow.

In 1931 two excellent strains of Yellow Globe Danvers that had been inbred for two generations were placed under a small cage. Two bulbs of each strain were planted, the plants were covered, and flies were added. When mature, the seed was harvested and planted. From one line was grown a population of 200 plants, of which only 10 individuals (5 per cent) were hybrids. From the other line was produced a population of 193 plants, of which 32 (about 16 per cent) were hybrids. In this case, the hybrids could not be selected in the seedling stage: the plants had to be grown to maturity, at which time the hybrids could be picked out because of their pronounced manifestation of vigor. The relatively small amount of crossing here can be explained, at least in part, by the fact that the flowering periods of these two strains do not exactly coincide, although they do overlap considerably.

In 1932 it was desired to secure a large number of hybrid plants between the varieties Crystal White Wax and White Persian, the latter being resistant to thrips. Under a cheesecloth cage, 6 feet square, were planted 11 bulbs of Crystal White Wax and 3 of White Persian. Flies were used for pollination. From the 3 White Persian plants was harvested 75 grams of seed; from the 11 Crystal White Wax, 200 grams. The White Persian variety has foliage of a very light-green color, which is recessive to the dark-green of Crystal White Wax. If seed of the White Persian is planted the hybrids can be selected in the seedling stage by the dark foliage color. When a portion of the seed from the White Persian was planted, the total population of seedlings was 264; of this number, 73 (about 27.5 per cent) were crosses.

In cases where the hybrid is not distinct, one parent will have to be emasculated. The umbels of the plants to be crossed are bagged as soon as the first flower opens. At first only a few flowers on an umbel open daily. The number increases until full bloom, when fifty or more may open in a single day. The flowers are removed each morning and afternoon, until about 50 open flowers can be secured in one or two days. If the weather is especially hot, they must be removed oftener, because the

anthers may shed their pollen very soon after the flower opens. Emasculation is conducted several times daily until about 50 emasculated flowers have been secured. Then all the buds are removed, a flower head of the pollen parent is tied with the emasculated head, and both are enclosed under the same bag (fig. 8). The bags are tapped several times each day to facilitate pollination, or, better still, flies are enclosed within the bag to do the pollinating. To secure pollen-free flies for crossing, pupae are placed in a paper plate, which is wrapped with cheesecloth

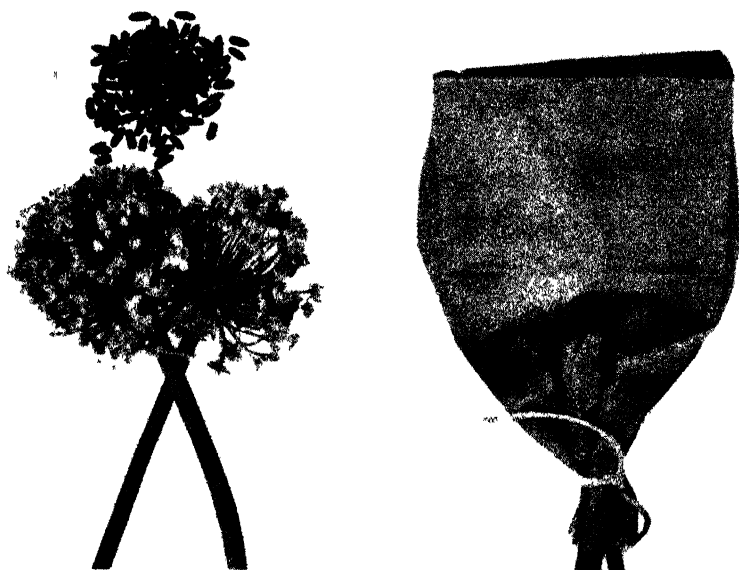


Fig. 8.—Method of tying umbels together for crossing. The one on the right has been emasculated; the other supplies the pollen. The umbels are then enclosed in a paper bag. Flies are enclosed to do the pollinating. Sometimes pupae are also included.

and set in a warm place. When the flies have emerged, they are taken to a low-temperature chamber (about 37° F). After chilling, they can be easily caught and placed in small glass vials, the corks being notched to permit ventilation. The adult flies needed for one bag are placed in a vial and are liberated in the bag at the time the two heads are tied together. The heads are bagged separately when pollination has been completed—usually 4 or 5 days after they have been tied together.

When plants to be crossed are not growing side by side, an umbel of the pollen parent can be removed and tied securely to the umbel that is to be pollinated; then the two are enclosed under a bag as in the method described above.

When flies or other insects are not used, the same method of emasculation is used; but pollination must be done by hand, at a time when the styles are fully extended. The pollen of the male parent can be collected on a watch glass, and the pollen-covered surface touched against the stigma of the female. As a rule, each flower must be pollinated several times in order to get even a fair set of seed.

Some investigators report that most of their crossing is done in the greenhouse. In the work reported here pollination was generally done in the field; but when the length of pollinating season had to be increased, plants have been brought to bloom in the greenhouse considerably earlier than out of doors. Conditions there are much more favorable for work; no wind or dew, and fewer insects, will interfere. Under Davis conditions, apparently, the best method for greenhouse pollination is to set the mother bulbs, late in November, in large pots which are sunk in the ground out of doors. Considerable root growth and some foliage development are made during the winter. About the time the seed stems are formed—usually late in February—the plants are removed from the pots and placed in a greenhouse bench. The higher temperature of the greenhouse usually brings the plants into bloom at least a month sooner than out of doors.

### HARVESTING AND CURING SEED

The bags are allowed to remain on the heads until the seed is ripe. Then the stems are cut below the bags, and all the heads of a plant are tied together and placed in well-aerated crates in the open until thoroughly dry. Next, the heads are placed in cloth bags, and the seed is

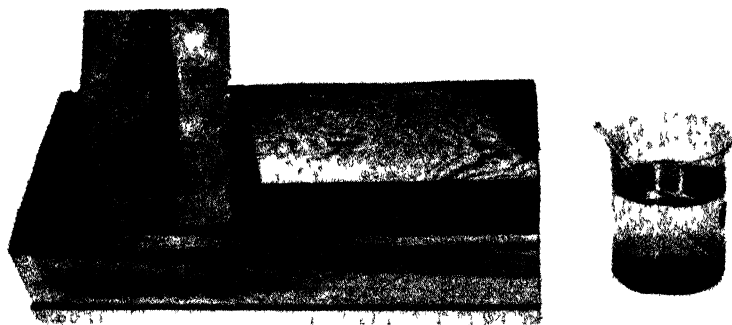


Fig. 9.—Rubber washboard used to thresh small lots of onion seed. After threshing, the seed and chaff are brushed into a beaker of water. The heavy seed sinks to the bottom, while the light seed and chaff are floated off.

rolled out. Probably a better method is to remove the seed by use of a rubber washboard (fig. 9). Seed capsules matured under a bag contain considerable nectar, which is rather hygroscopic, taking up moisture rapidly during humid weather and making threshing difficult. Threshing is most efficient during hot, dry weather. When well threshed, the seed and chaff are emptied into a container with water, and the light seed and chaff floated off. When single plants are being threshed separately, a 1,000-cc beaker is generally sufficiently large for washing. After three or four changes of water, the seed is usually clean and is spread thinly on paper to dry; it is then placed in small envelopes. A common mistake is to bag or package seed not sufficiently dry, causing it to mold.

### GROWING SEEDLINGS

For a number of years the onion seed of all the selfed lines of storage types was sown directly in the field. Although this method avoided the setback from transplanting, and cost less, it failed to provide a uniform stand of plants from which accurate comparisons could be made; it involved, furthermore, a great waste of seed. After several trials, accordingly, it was replaced by the transplanting method, which has since been used for all the breeding work.

There are two general seasons for seeding onions in this district. The nonbolting or Italian types such as California Early Red, Italian Red, Stockton Yellow Globe, and Giant White Italian Tripoli are sown in late August or early September. At Davis these varieties are always sown in coldframes and are usually transplanted to the field in November or December. About a month before sowing, the soil is sterilized with a formalin solution to kill the damping-off fungi and the pink-root organism, *Phoma terrestris* Hansen. The solution used contains one pint of commercial formalin to 15 gallons of water. One gallon of solution is applied to each square foot of soil. The bed is covered with burlap, wet down, and left for several days (fig. 10). When the soil is sufficiently dry it is worked into fine condition; then the seed is sown in shallow furrows, about 10 or 12 to the inch, and about 5 inches apart. Finally, the beds are covered with cheesecloth, which is left on both day and night until late October (fig. 11). Covering, in conjunction with the previous formalin treatment of the soil, prevents damage from the seed-corn maggot.

The storage varieties such as Australian Brown and Yellow Globe Danvers are usually seeded in coldframes in late November or December and transplanted to the field in February or March. Most of these varieties produce a high percentage of bolters if planted much earlier.

The soil in the coldframes is sterilized, and seeding is done as described above; but since the seed-corn maggot does no damage so late in the season, the beds are covered with a cloth only on very cold nights.



Fig. 10.—The seed beds are treated with a solution of formalin to kill the various fungus diseases in the soil. After the treatment, burlap is spread over the bed and is wet down to hold the fumes in the soil.

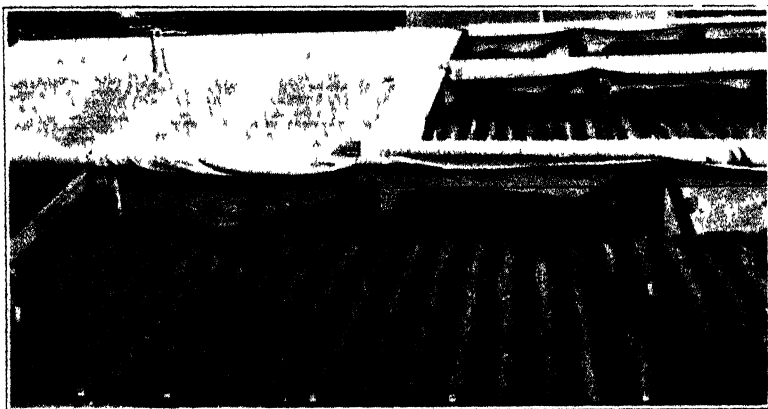


Fig. 11.—Growing seedlings under a cheesecloth cover to prevent attacks by the seed-corn maggot

### TRANSPLANTING

When sufficiently large, the seedlings are transplanted to the field (fig. 12). The large-growing Spanish and Italian types are set 4 inches apart in the row. On sediment soil where surface irrigation is practiced, the rows are made 18 inches apart; but on peat soil, which is subirrigated and where furrows are not necessary, the rows are 15 inches apart. The varieties that produce small bulbs are planted 3 inches apart in the row.

In the Sacramento Valley it is important to have large seedlings for transplanting and to get them into the ground early in the spring. In late February or early March, they are transplanted and kept alive without difficulty; but later, when the north winds begin and the days become longer and hotter, transplanting becomes increasingly difficult.

### OUTLINE OF BREEDING PROGRAM

The onion-breeding program that most concerns the seedsmen is that of purifying stocks. With most varieties this consists, mainly, in eliminating colors, shapes, and other characters not true to the variety. With many other crops, the method is to self a large number of desirable plants, grow the progeny, discard the off types, and continue this process



Fig 12.—Inbred lines and strains of onions are tested in single row plots, usually replicated three times

until the progeny are uniform for the desired characters. When continuous inbreeding is practiced with the onion, however, a gradual loss of vigor for a number of generations almost always results. This is the main disadvantage in attempting to purify onions by continuous inbreeding. Some inbreeding, however, is necessary if the most rapid progress is to be made. Lost vigor can be regained by crossing inbred lines.

The following plan, the result of our experiments to date, is suggested as the most suitable for the improvement and purification of onion varieties. Investigations now under way may later cause us to modify these recommendations somewhat.

*First year:* Select a large number of commercial bulbs that approach the ideal for the variety. The larger the number, the greater the chance of securing desirable lines. Plant for selfing.

*Second year:* Self-pollinate.

*Third year:* Grow progenies of each plant separately. Discard all lines that have a high percentage of off-type bulbs. Retain 25 or 30 of the best lines, and plant for selfing. These bulbs will probably be somewhat small, but size will be regained by crossing later on.

*Fourth year:* Self-pollinate.

*Fifth year:* Grow progenies of each plant separately. Discard all lines that are still segregating for important commercial characters. Retain at least 10 lines; from these select the best bulbs. It is necessary to retain a number of lines that have been derived from different bulbs at the first selection, because different lines must be crossed to regain the original vigor.

*Sixth year:* Group all selections and plant in the field so that the maximum amount of crossing will occur. Mass the seed.

*Seventh year:* Sow the seed. When the crop is mature, select large bulbs possessing the desirable characteristics for the variety; these result from desirable crosses.

In after years, stocks are maintained by selecting desirable bulbs and massing.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance given by L. C. Curtis, Vedder Wright, and Francis Winner during the course of these investigations.







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